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The Journal of Laboratory and Clinical Medicine

VOL. XIV ST. LOUIS, MO., AUGUST, 1929 NO. 11

CLINICAL AND EXPERIMENTAL

STUDIES IN THE ALIMENTARY TRACT OF MAN

I. THE ATTAINMENT OF RELIABILITY IN GASTRIC RESPONSES*†

BY T. WINGATE TODD, F.R.C.S. ENG., AND WILHELMINE KUENZEL, A.M.,
CLEVELAND, OHIO

INTRODUCTION

THE great improvement in roentgenoscopic methods during the past quarter century and the increasing employment of the technic for diagnostic purposes call for a new comprehensive study of the alimentary tract in its normal activity. This is emphasized by the many recent attempts to associate definite forms of stomach and dispositions of intestine with particular physical types of humanity. For such conclusions the evidence seems to us ill substantiated, and we do not propose to enter the discussion. It will be more satisfactory to make an inquiry of this nature after we shall have more precise information upon the norms presented by the alimentary tract in form, position and motility.

For our purpose the study must be made upon human beings, young and healthy, living under conditions which are controlled or at least definitely known. The observations must be repeated upon the same subjects until the possibility of chance effect is certainly excluded. It must not be an investigation which is the side issue of a professional examination of alimentary tracts known or suspected to be the seat of disorder.

For a carefully planned investigation of this kind the alimentary tracts of medical men themselves are obviously the most suitable. We have had the privilege of using those of our colleagues and students, as well as our own, in this analysis of anatomic expression of activity in the normal organs.

*From the Anatomical Laboratory, Western Reserve University, Cleveland, Ohio.

†Studies I-IV of this series form parts of a thesis for which the degree of A.M., was conferred by Western Reserve University upon Wilhelmine Kuenzel, June, 1928.

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In this and succeeding articles we propose to deal with the stomachs of one hundred and forty-seven young healthy adult males repeatedly examined by both roentgenoscopy and radiography under controlled conditions. It is the repeated examination which we seek to emphasize as most informative and the accumulation of radiograms of the same individual. Eliminating the numerous serial studies from which cinematographic records have been made, our average is seven radiograms per subject in addition to the detailed records of the fluoroscopic examinations.

TECHNICAL METHODS

Certain technical methods and principles of examination which can be standardized in advance should be given their validation, as it were, by checking possible errors. These can be quickly enumerated.

Barium Meal

While most of our original records were made after ingestion of a 16-ounce meal consisting of 4 ounces of barium sulphate in 12 ounces of buttermilk, we have definitely assured ourselves that a smaller meal is more satisfactory. In a later communication we shall present the evidence for this, but it may at once be stated that our standard meal is now one ounce of barium administered in four ounces of sweet milk at 70° Fahrenheit. This is given between two and three hours after a light meal, namely, breakfast or lunch, while the subject is, as yet, unfatigued by the day's work. It is neither necessary nor advisable to utilize a starved stomach. There is at least an equal likelihood of obtaining a complete stomach outline with a small meal of five ounces which permits more experiments in a short time and does not nauseate the subject.

Dosage

A 5-30 M.A. Coolidge tube, radiator type, with a 5-inch gap is used for both roentgenoscopy and radiography, 5 milliamperes being employed for the former and 30 milliamperes for the latter. The target distance for radiography is 36 inches, the exposure one to one and a half seconds, depending on the size and weight of the subject; and the tube is centered at the level of the disc below the second lumbar vertebra. Under no circumstances, even when a serial study is undertaken for cinematographic reproduction, is the total exposure permitted to exceed half a skin dose.

Tube Level

The close relation of stomach to vertebral column effectually prevents distortion from being registered in this relationship as a result of minor modifica-

TABLE I

INFLUENCE OF TUBE LEVEL

PATIENT	TUBE LEVEL	CARDIA	PYLORUS	GREATER CURVATURE
M.R.	U	MT12	UL4	Disc below L5
	L	MT12	Disc L3-L4	LL5
M.E.B.	U	ML1	LL4	1 cm. below L5
	L	UL1	LL4	1½ cm. below L5
W.M.K.	U	LT12	LL4	LL5
	L	LT12	LL4	ML5
T.W.T.	U	Disc T12-L1	LL3	UL4
	L	UL1	LL3	UL4

U = upper, M = middle, L = lower.

tions in tube level. In a large series involving frequent repetition of radiography on a single subject this is easily recognized. Table I is merely an example of the result obtained when the tube levels are deliberately two and a half inches apart. The discrepancy of one-third of a vertebra occasionally registered is no more than an expression of difficulty in reading levels on the film.

TABLE II

INFLUENCE OF CHANGE OF TUBE LEVEL ON VERTEBRAL COLUMN

	X1917 E TUBE CENTERED ON L3	X1917 F TUBE CENTERED 4 IN. HIGHER
Cardia	LT12	LT12
Pylorus	ML3	ML3
Greater Curvature	Disc L5-S1	Disc L5-S1

Table II gives the readings on a subject when the difference in the tube level was four inches. The readings were, of course, made without any idea of checking. It is but a chance culling from our records.

Errors

Stance of Subject.—Differences in vertebral curvature are the most fruitful source of discrepancy, the effect being naturally most pronounced by modification of lumbar curve. Modification in respiratory phase has a very minor effect.

Psychologic State of Subject.—The instability of stomach position in an untrained subject is easily demonstrated (see Routine Examination, page 1021).

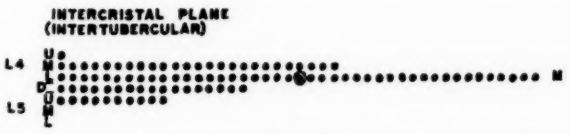

Chart I.—Site of intercristal plane in 96 male, White students. Vertebrae indicated in thirds: upper, middle, lower. Disc between fourth and fifth lumbar vertebrae also plotted. Median (encircled), mean (M) and mode all at lower L4.

Criteria of Position

External Features.—The vertebral column is the safest guide. Each lumbar disc corresponds roughly to one-third of a vertebra. Hence a vertebra and its subjacent disc form a group of four vertebral units. The intercristal plane is distinctly less useful because its level, relative to the vertebral column, varies (Chart I); its level, read on the fluoroscopic screen, is not precisely that shown on the radiogram; variations in stance affect the site of iliac crest shadow just as greatly as they affect the vertebral column. The umbilicus is practically constant in site at the level of the intercristal plane.

Gastric Features.—Vertical length. Maximum distance projected on the film in a straight line parallel with the vertebral column, from highest limit of Magenblase to lowest point on greater curvature. Cardia-pylorus and cardia-greater curvature lengths are also employed. Of these absolute dimensions one can make indices, but, like planimeter records of shadow area, they are of little value for computation of position since they must vary with the absolute dimensions of the subject himself. Fluoroscopic tracings are also of little value because the faint shadow is difficult to see with sufficient exactitude even

through the thinnest tracing paper practicable; it prolongs unnecessarily the exposure period; and respiratory movements, which cannot be eliminated even when the tracing is rapidly made, are reflected in a rise and fall of the stomach.

METHODS OF PRESENTING RESULTS

Charts are useful for showing the position of various parts of the stomach relative to the vertebral column. Tracings show size, shape and motility, as well as position. Planimeter records of area permit a comparison of relative size. Of course no one of these methods can be more accurate or dependable than the radiograms from which the records were taken. No one of them throws more than a very imperfect light on any problem. But in biology we are often faced with phenomena which result from the interaction of many diverse factors. And the only method of analysis may be the removal of one factor at a time. By this slow and tedious method we attempt to glean information from our records.

On the charts a vertebra is indicated in thirds, upper (U), middle (M), and lower (L). Beneath it is the disc (D). The intercristal (in practice the same as intertubercular) plane is taken in its modal position (ascertained on our series), namely, the lower third of the fourth lumbar vertebra.

For demonstrating actual changes in a stomach the most satisfactory scheme is the superposition of successive radiograms, but no person can hold himself in exactly the same posture at two successive examinations. Straightening of the lumbar curve withdraws the vertebrae from the film, and their shadows take on greater dimensions. Increase of the lumbar curve causes the vertebrae to approach the film, and their shadows consequently diminish in dimensions. Hence superposition of radiograms is not so easy in practice as it is in theory. Again, the pelvis is often slightly tilted to one side or the other. And thirdly, the arrest of diaphragmatic movement may have occurred in slightly different phases of the respiratory cycle. If the subject be instructed to inhale deeply and then to exhale until his chest reaches the position of greatest ease, he will usually stop his diaphragm in the same or practically the same position each time. The inclination of the rib shadows on superposition permits estimation of the error, if any, from this cause.

The tracing is not an accurate indication of the vigor of gastric activity, and one must not depend upon it for an estimate of the amplitude of peristalsis. It is at best a chance momentary representation of a continuous action; and, indeed, it may show no indication whatever of peristalsis, for sometimes the very technic of radiography may temporarily inhibit gastric peristalsis. It is on the fluoroscopic study that we must rely for accurate information on peristalsis.

As for planimeter records, it may be objected that area is no criterion of volume. The directions in which a stomach enlarges are, however, quite definitely those of the longitudinal and circular muscular fibers, lateral distention taking place along the greater, never along the lesser, curvature. In spite of careful search we have never seen the stomach enlarge in its anteroposterior dimension in comparison with its enlargement in transverse or longitudinal dimensions (see Influence of Occupation, page 1025).

Considerable experience is required to determine with assurance on a radiogram the outlines of cardia, pylorus, lesser curvature and sometimes of gastric tube even after administration of a meal. This is not due to the smallness of the meal; the pylorus may be quite indefinitely delineated on the radiogram even after one has observed, by means of the fluoroscopic screen, barium passing into the duodenum. The sites of cardia and lesser curvatures are still more difficult to define, and resulting errors affect the planimeter record. Six or seven per cent of error in area must be allowed for (see Influence of Occupation, page 1025). In spite of these defects the planimeter record is a useful method of comparison for a particular stomach at successive examinations, and with reservations it may be usefully employed in the comparison of different stomachs, especially if the subjects be young, about the same age, comparatively slender and of fairly uniform build.

ROUTINE EXAMINATION

All our subjects have been examined between two and three hours after a light meal, breakfast or lunch. We find that by this time the stomach is empty except for the negligible residuum which is apparently normal to some people (see Physical Condition of Subject). It is an essential feature of our study that the stomach should be empty but not starved.

Although studies have been made of our subjects in both vertical and horizontal postures, we confine our attention to the former unless it is specifically otherwise stated.

By administering the experimental meal in different amounts to the same subjects we found that size of meal bears no direct relation to the exact size or precise position of the untrained stomach, and even on the trained stomach it is quite without effect upon gastric shape. The untrained stomach does not adjust itself systematically to the exact amount of barium contents and is usually practically its original size after much contents have passed through the pylorus.

A fundamental necessity of all roentgenologic examinations is the quiet, orderly carrying out of the technical procedure. Any confusion or irregularity in the precise arrangements or even uncertainty in the minds of those responsible is at once transferred to the subconscious ego of the subject and is reflected in his alimentary response. Every detail of our technic is therefore carefully rehearsed by the staff before the actual session commences.

PHYSICAL CONDITION OF THE SUBJECT

It is not enough to safeguard one's observations by carefully controlling the methods of examination. The physical condition of the subject and his psychic state must also receive careful attention, and control must be established over these features of the work.

The antiquated preliminary routine of purgation and starvation itself had profound effects on the subject both physically and psychically. Purgation is no longer required because of increased penetrating power of the radiographic apparatus. Neither is starvation necessary to empty the stomach. The subject should suffer no interruption of his daily habits. The lapse of between two and

three hours after a previous light meal such as breakfast or lunch is enough to ensure an empty stomach. If, as happens in some people, a small residuum of contents remains, it is expelled at the moment of or quickly after ingestion of the new meal.

Time of Day

Since circumstances compel us to examine freshman students in the afternoon and sophomores in the morning, it is necessary to inquire whether any differences found in gastric behavior may be due simply to the time at which examination is made. To make a critical investigation of this problem on a large scale is impracticable; the evidence must be obtained by chance. We have, however, experimented with stomachs of members of the staff at all times, the same stomach being examined in early morning, at noon and in the afternoon. We have failed to find any evidence of real difference in response except in the late afternoon of a strenuous day. This difference is plainly due to fatigue and is not relevant to the present problem. On the basis of our staff experiments we believe it permissible to compare morning and early afternoon records. This decision is supported by our finding that the February afternoon records of freshmen are intermediate in type between the October afternoon freshman and October morning sophomore records (see Possible Influence of Season, page 1030).

Effect of Body Posture on Stomach Position

To investigate this problem a group of students in the latter part of their freshman year submitted themselves for examination. Each was studied in both vertical and horizontal postures. Two radiograms were taken successively with the subject standing erect and lying on his back. The results are given in Table III and Chart II. Cardia, pylorus and greater curvature all undergo definite modification in site, though individual variation in the extent of this modification is considerable. Cardia is the least affected, and its change in position may be inseparable from modification in precise position of the diaphragm induced by change in posture. Concerning this we

TABLE III

CHANGE IN STOMACH POSITION WITH CHANGE FROM VERTICAL TO HORIZONTAL BODY POSTURE

PATIENT	DATE	CHANGE IN SITE IN VERTEBRAL UNITS		
		CARDIA	PYLORUS	GREATER CURVATURE
203	Jan., 1925	4	5	7
204	Jan., 1925	3	5	10
208	Jan., 1925	4	11	12
212	Jan., 1925	6	?	13
214	Jan., 1925	2	6	8
215	Jan., 1925	4	7	10
215	Oct., 1925	1	4	7
217	Jan., 1925	0	7	6
224	Jan., 1925	?	6	7
227	Jan., 1925	1	4	5
227	Oct., 1925	0	6	8
229	Jan., 1925	0	5	7
229	Oct., 1925	4	10	11
	Total (9)	29	(12) 76	(13) 111
	Average	3.2	6.3	8.5

have at present no data. The cardia is elevated 3.2 vertebral units, that is to say, about the height of one vertebra. The pylorus is raised approximately twice and the greater curvature almost three times this amount. Since these figures are obtained from the record of the number of vertebral units through which the gastric features rise in each individual, we feel they give a truer picture than does the chart which registers the differences between average positions of the respective gastric features before and after change of posture. By the chart the cardia rises two units, the pylorus six and the greater curvature ten.

In spite of modification in position of the three chief sites of the stomach we must emphasize the fact that the essential shape of the stomach does not change in corresponding manner. The proof of this contention lies in exami-

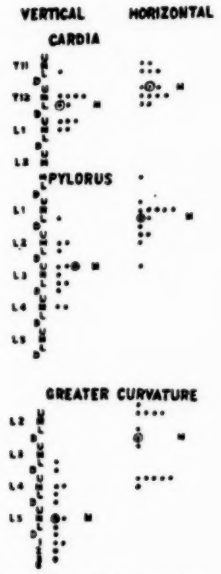

Chart II.—Level of stomach sites in 14 male, White students. Vertical and horizontal postures. Indicators as in Chart I.

nation of the radiographic records themselves, and we can present merely one illustrative instance (Fig. 1). Student No. 215 was examined in January, 1925, and in the following October. During this interval the general gastric contour altered so that in October the stomach was more obliquely placed than in January. In the change from vertical to horizontal posture this essential difference in gastric contour is maintained. Now it must be remembered that this stomach (No. 215) was not a mere casual untrained organ. One cannot too often insist that the stomach of unpredictable response is that of the casual patient or untrained subject.

Relation of Change in Weight to Stomach Position

In making examinations involving long periods of time the problem of change of weight sometimes obtrudes itself quite emphatically and must therefore receive attention. Now the fluctuation in weight during a single

day, as shown by our staff records, may amount to or even somewhat exceed four pounds. Hence we have ignored changes in weight of less than this amount. These changes in weight may have developed during a period of six or of twelve months; the lapse of time is unimportant.

In the series utilized there were fourteen students whose weight had increased by an average of 7.3 pounds, and ten whose weight had decreased on the average 5.6 pounds. In the series with increased weight the total rise in position is recorded for the three chief sites of the stomach, having regard to sign. For the cardia this amounts to eleven vertebral units, for the pylorus twenty-seven and for the greater curvature fifty-six; approximately an average of one vertebral unit (or one-third of a vertebra) for the cardia, two for

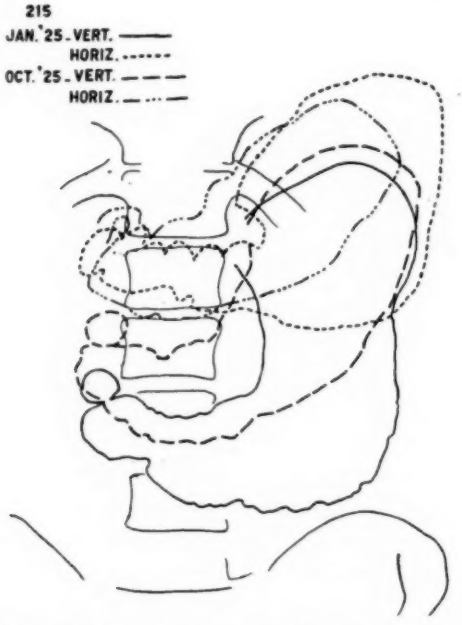

Fig. 1.—Effect of body posture on stomach position. Change from vertical to horizontal posture is accompanied by alteration in levels of stomach sites but not in essential characters of stomach outline.

pylorus and four (one vertebra with disc) for greater curvature. There is great individual variation and no uniformity in change of level. As the results appear so equivocal it would be quite justifiable to hold that some factor other than increased weight is responsible for the change in position even of the greater curvature.

In this uncertainty we turn to the second part of the table. The average diminution in weight of the ten students in the other group is 5 to 6 pounds. The total lowering of cardia, having regard to sign, is ten units, of pylorus twenty, and of greater curvature ten. In our routine work we should be disinclined to pay much heed to a change in position averaging for cardia and greater curvature one unit each and for pylorus only two units. But the lowering with loss of weight and raising with gain in weight occur far too uniformly for us to dismiss them as pure chance, especially since it is by no

means necessary to postulate a direct or proportional relationship between change in weight and modification of stomach site. Our results do at least seem to bear out the observation that small highly placed stomachs occur in the healthily corpulent and low placed stomachs in the thin, emaciated and enfeebled.

TABLE IV

1. CHANGE IN STOMACH POSITION WITH INCREASE IN WEIGHT

PATIENT	INCREASED WEIGHT	CHANGE IN SITE IN VERTEBRAL UNITS		
		CARDIA	PYLORUS	GREATER CURVATURE
1005 A E	+ 4 lb.	+ 1	+ 1	+ 4
1006 A D	+11¾	+ 3	+ 4	+ 7
1009 A E	+ 4½	0	− 2	+ 3
1010 A D	+ 4¾	− 2	− 3	− 1
1024 A D	+ 8	− 4	− 2	+ 3
1025 A D	+ 4¾	+ 1	+ 3	+ 4
1029 A E	+10	0	0	0
1037 A D	+ 4	− 2	+ 5	+ 4
305 A I	+19¼	+ 1	− 2	+ 6
308 A G	+10	+ 5	+ 3	+ 5
309 A G	+ 4	+ 7	+ 9	+ 7
312 A G	+ 6	0	+ 1	+ 3
317 A G	+ 7	+ 3	+ 7	+ 7
332 A G	+ 4	0	+ 3	+ 4
Total 14	Av. + 7⅓ Total	+11	+27	+56

2. CHANGE IN STOMACH POSITION WITH DECREASE IN WEIGHT

PATIENT	DECREASED WEIGHT	CHANGE IN SITE IN VERTEBRAL UNITS		
		CARDIA	PYLORUS	GREATER CURVATURE
1017 A E	− 8 lb.	+ 1	− 7	− 7
1021 A E	− 4	0	0	0
1023 A E	− 5¾	− 3	− 3	− 3
1026 A E	− 7¼	− 4	− 2	− 1
1030 A D	− 4½	− 1	− 2	− 3
1033 A D	− 6½	− 2	− 1	− 1
311 A G	− 7½	− 2	0	− 2
303 A G	− 5	+ 1	− 2	0
330 A G	− 4	0	− 3	+ 3
336 A G	− 4	0	0	+ 4
Total 10	Av. − 5¾ Total	−10	−20	−10

Since the pylorus is much less susceptible than the greater curvature to direct pressure by other abdominal organs, the change in position of pylorus has a greater possible significance.

We must now consider certain conditions which may affect the subject but are even less easily segregated in their influence than those we have studied.

Influence of Occupation

It is a well-known observation that the general physical condition is reflected throughout the systems. The occupation of a student, therefore, may only be considered to affect his alimentary tract in so far as that occupation affects his general health.

In two groups of our October sophomore series we have taken planimeter records of area from tracings made of stomach radiograms, separating the records according to occupation of the students.

To the first group were given 16-ounce buttermilk meals. Of these students thirteen had been occupied during the summer in indoor work such as hospital laboratory technicians, night clerks and workers in machine shops. Twenty-two came from outdoor occupations such as road engineers, camp counsellors and farmers. The complete observations are set forth in Table V. Crude average area of stomach shadow determined from tracings of radiograms made ten minutes after ingestion of the meal is 20806 sq.mm. for indoor workers and 18836 sq.mm. for outdoor workers. The difference between these figures, namely 1970 sq.mm., somewhat exceeds 10 per cent of the outdoor average.

To the second group 5-ounce buttermilk meals were given. The crude average area of the eight indoor stomach shadows is 11797 sq.mm. whereas that of the nine outdoor stomachs is 12287 sq.mm. This time the difference, 490 sq.mm., is slightly more than 4 per cent of the indoor average.

TABLE V

AREAS OF STOMACH SHADOW

16 OUNCE MEALS

INDOOR OCCUPATION PATIENT	AREA	OUTDOOR OCCUPATION PATIENT	AREA
221F	14960 sq. mm.	201F	18060 sq. mm.
227E	23560	202E	17770
228F	18180	204E	14700
229E	19700	205E	18800
230E	22900	207F	19430
303G	20260	213F	16440
307G	21200	215E	16230
308G	24390	224E	16580
315G	20620	225F	19900
328G	27050	305I	19050
329G	17880	306G	18330
331G	21450	309G	20100
333G	18330	310G	20950
		312G	24750
		318G	22740
		320G	21400
		321G	17530
		327G	21790
		330G	17300
		332G	17200
		335G	19250
		336G	16100
Average of 13	20806	Average of 22	18836

5 OUNCE MEALS

INDOOR OCCUPATION PATIENT	AREA		OUTDOOR OCCUPATION PATIENT	AREA	
	1	2		1	2
214E	12040	11050 sq. mm.	1003D	16200	18100 sq. mm.
314M	11990	12000	1006D	11220	11700
1005E	10730	10450	1008D	14630	15800
1023E	12030	12170	1009D	10200	10190
1028E	10350	10100	1018E	10910	11450
1030D	14060	15260	1024D	8190	8350
1033D	12220	10400	1031E	14250	14900
1034D	10960	11000	1035E	14520	14500
			1037E	10460	12290
Average of 8	11797	11554	Average of 9	12287	13031

Since the outdoor average was the smaller in the first group and slightly the greater in the second, we must presume that the differences are due to technical errors and not to occupation. Hence we made a second tracing of each of the 5-ounce series and have added the records to Table V. For the outdoor group the difference in average area from the two records is 744 sq.mm. Hence differences which amount to less than 6 per cent of the total area to be compared must not be considered as significant (see Methods of Presenting Results, page 1020).

Now the 5-ounce series easily falls within the 6 per cent probable error. This makes us doubt the wisdom of reading significance into the difference in the 16-ounce series. It may be that the larger amount of contents predisposes the record to a larger error. In the 5-ounce group the difference of average areas in our second set of tracings is 1477 sq.mm., almost 13 per cent of the smaller average which happens to be that of the indoor stomachs.

If there be any difference in stomach area related to occupation, it must be sought and proved by other means than the one here adopted.

Incidentally it should be mentioned that we have found no way of checking anteroposterior depth of barium shadow. Our observations during the fluoroscopic examination assure us that it is much less subject to change than the transverse or longitudinal diameters, but it would be absurd to

TABLE VI

1. EFFECT OF INDOOR OCCUPATION ON STOMACH ACTIVITY

No.	Sudden Distention	Sudden Elongation	Peristalsis	Passage Seen
5-Ounce Buttermilk Meal on Empty Stomach				
314M	None	None	Immediate	Immediately
1030D	None	None	Immediate	Immediately
1033D	After 1 min.	None	After 2 min.	Immediately
1034D	None	None	Immediate	Immediately
5-Ounce Buttermilk Meal 1 Hour After Milk Meal				
214E	Immediate	Immediate	After 1 min.	Immediately
1005E	Immediate	Immediate	After 1½ min.	After 2 min.
1023E	After 5 min.	None	Immediate	After 5 min.
1028E	None	None	After 2 min. pulsation After 5 min. waves	Immediately

2. EFFECT OF OUTDOOR OCCUPATION ON STOMACH ACTIVITY

5-Ounce Buttermilk Meal on Empty Stomach				
1003D	Immediate	Immediate	Immediate pulsation. After 4 min. waves	After 4 min.
1006D	After 1 min.	After 1 min.	Immediate	Immediately
1008D	Immediate	None	After 2½ min.	Immediately
1024D	After 2½ min.	None	After 8 min.	After 1 min.
1037D	None	Immediate	After 3½ min. shimmer	After 4½ min.
5-Ounce Buttermilk Meal 1 Hour After Milk Meal				
1009E	Immediate	None	Immediate	Immediately
1018E	None	None	Immediate	Immediately
1031E	None	Immediate	Immediate	Immediately
1035E	None	None	Immediate	Immediately

contend that there is no alteration (see Methods of Presenting Results, page 1020).

Area of shadow, however, is but one criterion of gastric activity. One must also consider the vigor of peristalsis, and this cannot be deduced from the radiogram (see Methods of Presenting Results, page 1020). Extracts from the verbal records of the fluoroscopic examinations are submitted as Table VI. Lest an unforeseen difficulty be introduced, we have separated each series into two groups: in the first, the buttermilk (5 ounce meal) is administered on an empty stomach; in the second it is given one hour after a similar meal of sweet milk. The reason for adopting these alternative methods of exhibition of buttermilk is explained in our forthcoming article on the gastric response to milk and buttermilk. It is apparent from Table VI that any differences in response are bound up with some obscure factor in the individual himself and not due to occupation or physical condition of the stomach. We are therefore now in a position to make this further analysis of the subject himself.

The Group Response

In considering the results presented in this section of our study, there occurs the thought that part, at least, of the differences observed in the October and February records may be seasonal in character. The investigation would then be incomplete without some consideration of this possibility.

In spite of the fact that we have been unable to discern any essential difference between the gastric responses of sophomores when investigated in their relation to occupation, it is equally certain that an improvement in gastric response is observed in sophomores generally; and inasmuch as this appears fairly uniformly in the group and not erratically with great individual discrepancies, we will temporarily define it as the group response. In order to render accessible the data upon which this conception is based we have arranged the planimeter records of successive groups of students in Table VII.

In the first part of this table are the average records for freshmen and sophomores made from tracings of radiograms taken ten minutes after the barium meal. The average reduction in area from freshman to sophomore years in 1925 was 3382 sq.mm. It should be noted that the freshman records in this group were made in February, not in October, of the freshman year. They were the first records of students made in this investigation. It is, of course, impossible to decide whether the smaller average area in the student as a sophomore is due to more rapid emptying or to a smaller increase in size on swallowing the meal than is characteristic in freshmen. At the moment we are concerned with the record of size alone.

The next group of students was examined at the beginning of their freshman and sophomore courses in October 1925 and October 1926. In addition they were examined in February 1926. The decrease from October to October was only 2379 sq.mm., and that from February to October 2450 sq.mm. Let us ignore for the moment the fact that the change is equally great from February to October as from October to October, and also the

fact that the reduction in area in this group is less than that shown in the first group. The important fact is that there is a reduction.

Another phase of this same investigation is shown in the second part of Table VII where we observe the reduction in area of stomach shadow between the taking of the 10-minute and 60-minute radiograms after the meal. In the freshmen of February 1925 the reduction of area is 4202 sq.mm. In the same stomachs the following October the reduction is 4442 sq.mm. In the succeeding group, the freshman-October diminution was 3916; the freshman-February diminution was 4823; the sophomore-October reduction was 5760 sq.mm.

TABLE VII

COMPARISON OF FRESHMAN AND SOPHOMORE AREAS

RECORDS FROM RADIOGRAMS TAKEN 10 MINUTES AFTER MEAL				
		Year	Average Area	Difference
Class of 1928				
Freshmen	(A_1)	Feb., 1925	21545 sq. mm.	
Sophomores	(A_2)	Oct., 1925	18163	3382 (A_1–A_2)
Class of 1929				
Freshmen	(B_1)	Oct., 1925	22829	
Freshmen	(B_2)	Feb., 1926	22900	2379 (B_1–B_3)
Sophomores	(B_3)	Oct., 1926	20450	2450 (B_2–B_3)

RECORD OF REDUCTION IN AREA FROM 10 MINUTES TO 1 HOUR AFTER MEAL				
	Year	Av. 10 Min. Area	Av. 1 Hr. Area	Difference
Class of 1928				
Freshmen	Feb., 1925	21545 sq. mm.	17343 sq. mm.	4202 sq. mm.
Sophomores	Oct., 1925	18163	13720	4442
Class of 1929				
Freshmen	Oct., 1925	22829	18913	3916
Freshmen	Feb., 1926	22900	18077	4823
Sophomores	Oct., 1926	20450	14690	5760

The second part of the table, detailing the reduction in area during the hour, perhaps points to the inference that the reduction in area when freshman and sophomore 10-minute radiograms are compared is due rather to a more rapid emptying than to a smaller initial increase in size. Whichever be the true explanation, the striking fact remains that a significant decrease is noted. Some students exemplify this better than others, but it occurs in the group as a whole and is associated with progressively increased experience and more understanding cooperation. In other words, it is associated with an increasingly stable and controlled central nervous reaction. We believe that any experience or condition of life which increases the stability and control of the central nervous system improves also the gastric response in the manner just indicated. While we have been unable to determine from quantitative analysis any relative difference in improvement as between freshman and sophomore responses which can be related to the type of life which the individual has been living, we still feel that the gastric response of a student who has been leading a healthy and wholesome out-of-door existence is better than that of a student occupied in indoor or dismal and uncongenial surroundings.

The Possible Influence of Season

The first part of Table VII shows that reduction in area of the 10-minute radiogram is to be found only in the sophomore stage: there is no diffeernce between the average areas of stomach shadow recorded in October and February of the freshman year. Nevertheless the 60-minute radiograms demonstrate a more marked reduction in area of shadow during the hour in February when the students have grown accustomed to our routine. It came as a surprise to us to find that the 10-minute radiograms showed an average area as large in February as in the previous October. The group response which upon the basis of the previous year's work (Table VII, A_1, A_2) we felt sure would be evident, and which indeed is evident in the 60-minute radiograms, is utterly lacking in the 10-minute ones.

There are two interpretations possible from these records. Either increased gastric vigor in the February records shows itself as more rapid emptying which is tardy in its full development, or there is some influence inhibiting the stomach from that immediate activity characteristic of the October sophomore. If the former explanation hold good we might expect to find progressively improved gastric activity in the February examinations as the years pass and the tradition of this method of instruction becomes better established. We have just completed the spring studies of the 1931 class and although the records are not yet in order for quantitative analysis, we find, to our disappointment, that no obvious improvement is evident over the records obtained of the same stomachs last October. Apparently then the group response cannot be depended upon to act early after ingestion of a meal in February of the freshman year. However, since it is obviously present in the 60-minute radiograms, there must be some factor inhibiting its expression at first. Further, so far as we are able to judge, this factor is equally as potent now as it was two years ago. The possible factors which one might invoke are occupation and season. After a few months of the confined and close study required by the medical curriculum it might be expected that the gastric response would be modified. But we have already presented our data upon this score and have failed to demonstrate quantitatively any effect of occupation upon gastric response.

The factor which we have not yet considered is that of season. It would be reasonable to assume that even all the varied influences which can be grouped under the term season would be able to curb but imperfectly the improving gastric response. As a working hypothesis we present this aspect of our study in the expectation that opportunity will occur for later and fuller investigation. Such scraps of information as we have been able to elicit from our observations on stomachs of members of the staff and other chance opportunities presented to us, all point in the same direction. They suggest that gastric activity is more sluggish during the winter months, and that most uniform responses are to be elicited after the health-giving, greater freedom of the summer.

It must be noted that the term season is being used in a very broad sense, covering all living conditions, including diet, reduced bodily activity, occu-

pational conditions, lack of sunshine and fresh air, which are regularly associated with winter in this latitude.

CONCLUSION

It is at this stage of the investigation that we find solid ground beneath our feet for the first time. The fault from which all previous work on the alimentary tract suffers is the overlooking of the necessity to use only trained stomachs under adequately controlled conditions. Failure to secure this essential basis is responsible for the bizarre, conflicting and equivocal results hitherto obtained. From the day when this secret was revealed to us our investigations have taken on a new directness, and the results have assumed that orderliness which one may expect from a properly controlled physiologic experiment. It is for this reason that we have developed the argument in this article up to the stage of the group response. In further communications it is possible to present the results of our studies upon the normal motility of the human stomach.

This article is a record of our effort to find a secure basis upon which to build a new study of the normal motility of the human stomach.

It is clear that uniform, controlled or known conditions must be secured with far greater certainty than has hitherto been attempted before results of any real value can be obtained.

We have made it our business to investigate critically each step of the necessary procedure and have ultimately discovered in the subject himself the source of error which has constantly vitiated all previous work. Every other factor can be allowed for except this. But the reaction due to this factor is incalculable. The subject must be willing, capable of entering intelligently into cooperation with the observer, and he must be trained so that the subconscious effect upon him of the roentgenologic examination is at a minimum. Inadequate appreciation of the essential nature of this precaution will certainly lead to disaster.

In our investigation of the problem we have standardized our technic and our methods of presenting results. We then set out to standardize our subjects, controlling time of day, body posture, weight, occupation and season of year. It is after a full year's training in roentgenologic method and general medicine that the student first becomes reliable as a subject. Until that date his reactions cannot be predicted with any assurance. Hereafter we shall demonstrate how easily they may be upset even when once established.

SUMMARY

1. An investigation of the human alimentary tract which does not involve standardized technic, controlled conditions of experiment and subjects carefully trained in roentgenology, is nowadays without value.

2. The old large meal on a fasting stomach is less likely to give a truly normal result than a small meal upon an empty but not starved stomach.

3. The close anatomic relationship between stomach and vertebral column in the living makes the latter the best indicator of gastric position. The exact level of x-ray tube is of little importance in determination of site of gastric points.

4. Planimeter records of area are to be accepted with reserve, for they may result in considerable error if used to indicate gastric volume. Properly controlled and used as averages of series they can be quite helpful.

5. Time of day within reason has no effect upon gastric response. Time of day which really involves fatigue has a marked effect. Season of year probably has an effect, although an indirect one.

6. Alteration of body posture influences levels of gastric features but not essential characters of gastric contour.

7. Change of body weight seems capable of an indirect influence on gastric shape exerted through the general well-being of the subject. Occupation acts in the same manner.

8. The culmination of our work lies in the demonstration of the influence of training of the subject upon constancy of gastric response. Further studies amplify this fundamental necessity of all gastric investigations.

CORYNEBACTERIUM ULCERANS: ITS EPIDEMIOLOGIC IMPORTANCE*

By Ruth Gilbert, A.M., M.D., and F. Constance Stewart, M.S., M.D.,
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PATHOGENIC microorganisms resembling diphtheria bacilli in morphology are always of interest and especially when they are found in lesions of the throat, since the clinical manifestations may be attributed to diphtheria and microscopic examination of cultures may offer no assistance in determining the etiology of the infection. We have been studying a species of such microorganism for which the name C. ulcerans has been proposed. Even though cultures of these bacteria are not encountered frequently, they have been found in specimens submitted from widely separated parts of the state.

As the species has been previously described,[1] only the characteristics which have seemed most important need be mentioned.* In young cultures, after from four to eight hours' incubation, these microorganisms have the morphology of diphtheria bacilli. In older cultures, following from eighteen to twenty-four hours' incubation, the majority of the cells are coccoid. More of the bacillary forms may be found, however, in mixed cultures after the longer incubation period. Culturally, this species resembles the diphtheria bacillus, except in its ability to liquefy gelatin and its failure to reduce nitrates. One of the most striking things concerning the microorganism is the extremely slight protection which diphtheria antitoxin affords animals inoculated with

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the culture or its soluble toxin. When two guinea pigs, one of which has received 500 units of diphtheria antitoxin, are given intracutaneous injections of this material, a similar reaction is induced in both animals, i.e., marked induration, congestion, and necrosis usually followed by the formation of an ulcer. After subcutaneous inoculation, a large ulcer develops and occasionally the animals die. At autopsy, the lesions are found to differ in some respects from those induced by diphtheria toxin, especially in that intense, diffuse, hemor-

Fig. 1.—Four-hour culture of C. ulcerans on Loeffler's blood-serum medium, demonstrating polar bodies. Albert's stain. X2000 diameters.

Fig. 2.—Four-hour culture of C. ulcerans on Loeffler's blood-serum medium, showing banded forms. Albert's stain. X2000 diameters.

rhagic infiltration of the adrenals is absent. Like diphtheria bacilli, the microorganisms remain localized. They have not been found in the heart's blood.

Owing to the marked changes in morphology which occur during incubation, the presence of C. ulcerans in throat cultures is seldom recognized if a morphologic examination only is made. When bacillary cells are found, the cultures are thought to contain diphtheria bacilli or organisms resembling them, while the short forms may be mistaken for cocci.

In the past four years, there have been three outbreaks in which the clinical symptoms have in some cases simulated diphtheria. C. ulcerans has been

associated with these infections, although the presence of diphtheria bacilli could not be definitely excluded. From September 4 to 29, 1925, 16 cases of diphtheria were reported in the vicinity of a county laboratory.* Although the clinical symptoms were not considered entirely typical, the appearance of the throat warranted the administration of antitoxin. No diphtheria bacilli were found in most of the first cultures, but they were reported in many of the second. In an attempt to learn the reason for unsatisfactory results, the bacteriologist in charge of the laboratory collected a series of cultures himself and examined them with equally irregular results. None of the organisms associated with Vincent's angina was recognized. Furthermore, when cultures from some of the patients were examined in a near-by city laboratory, no diphtheria bacilli were found.

One of the cases which occurred in the epidemic proved fatal. Four days before death, a physician saw the patient and prescribed a gargle. A culture was not taken. The day before death, another physician was called. At that time there was a membrane extending over the uvula and soft palate and into the mouth. A diagnosis of diphtheria was made, 12,000 units of antitoxin were administered, and a culture was taken. Prior to the death of the child on the following day, an additional dose of 18,000 units of antitoxin was given. The culture from this patient was not found to contain diphtheria bacilli, but in a transfer from it microorganisms having the morphology of diphtheria bacilli were seen. When an attempt was made to secure these bacteria in pure culture by the inoculation of rabbit-blood agar in plates, all of the colonies fished were thought to contain cocci. Transfers from two of these colonies on Loeffler's serum medium, however, contained what appeared to be diphtheria bacilli. When medium in plates was inoculated with them, the bacteriologist could find nothing but coccus-like forms in the colonies studied and unfortunately the cultures were then discarded. As twelve of the patients had used milk from one dairy, cultures were obtained from all of the milk handlers. Microorganisms having the morphology of diphtheria bacilli were found in one of these cultures, which was from M. J., a schoolboy who worked part-time at the dairy, washing bottles and occasionally helping to cap them. No history of sore throat or other illness could be obtained from him. In attempting to isolate the bacillary forms in the culture from this boy, the bacteriologist encountered the same difficulty that he had experienced in studying the culture from the fatal case. Another specimen was then collected from M. J. and submitted to the laboratory in Albany. It was found to contain C. ulcerans. Although there is no definite proof that this species was the etiologic factor in this epidemic, the description of the laboratory findings strongly suggests that the organism was present in some instances at least.

There have been two other groups of cases in which C. ulcerans has appeared to have definite epidemiologic importance. In the first group, this microorganism was found in the throat cultures of five people working on a dairy farm. After examination of the first culture, the milk was very promptly pasteurized and the infection did not spread further. On June 14, 1926, a man consulted a

*Information relative to this epidemic was received from Dr. J. P. Garen, formerly in charge of the Cattaraugus County Laboratory, Olean, October 19, 1925.

physician concerning an infected finger. The following day he complained of sore throat and in the culture taken at that time microorganisms having the morphology of diphtheria bacilli were present. When a subsequent culture was submitted for a virulence test, it was found to contain C. ulcerans. The clinical symptoms in this case lasted only four days. Cultures from four of his associates, none of whom had complained of sore throat, contained the same species of microorganism.

Fig. 3.—Four-hour culture of C. ulcerans in infusion broth medium, showing clubbed forms. Stained with Loeffler's methylene blue. X2000 diameters.

Fig. 4.—Forty-eight-hour culture of C. ulcerans on blood agar, showing coccoid forms. Stained with Loeffler's methylene blue. X2000 diameters.

In the third group of cases, the infectious agent was probably derived from milk. On July 9, 1926, J. P., a boy of eleven years of age, who helped strain the milk on his father's dairy farm, developed a sore throat. Although only a small amount of exudate was present on his tonsils, he was given 3,000 units of diphtheria antitoxin. No membrane was noted in his throat on the next day, but he stopped handling the milk at that time. C. ulcerans was found in the cultures submitted from this patient and persisted for about five weeks. From June 30 to July 31, cultures were sent for laboratory examination from 23 people who, with one exception, had used milk from the dairy farm on which J. P. was working. All had complained of sore throat. In

many of the cases, a membrane was present on the tonsils and antitoxin was administered. The symptoms lasted usually from two to three days only. C. ulcerans was found in the specimens from eight of these patients, but microorganisms with the morphology of diphtheria bacilli were not found in the cultures from the others. Only morphologic examinations were made unless bacillary forms were seen in the cultures. Hence some of them may have contained C. ulcerans which had assumed the coccoid form. From the dates of onset of the various cases, it seems probable that there was more than one carrier among the milk handlers, or that some of the patients had contracted the infection from previous cases.

It will be noted that these groups of cases have been associated with milk supplies. Most of the patients from whose cultures C. ulcerans has been isolated have lived in rural districts, and not infrequently have been milk handlers. The possibility of these microorganisms being derived from lesions in domestic animals, especially cows, is thus suggested. Although cultures of C. ulcerans have not been found to correspond exactly with the description given of any of the pathogenic diphtheroids that have been isolated from animal lesions, they resemble in many respects C. pseudotuberculosis of Preisz.[2] A comparative study of the immunologic properties of this species and other strains that have been found pathogenic for animals is therefore indicated.

SUMMARY AND CONCLUSIONS

The close resemblance of Corynebacterium ulcerans in young cultures to the diphtheria bacillus, and its coccoid appearance after longer incubation, make animal inoculation necessary for identification. Since most throat cultures receive a morphologic examination only, the prevalence of infections due to C. ulcerans may be greater than past experience has indicated. It is desirable to consider the possible presence of this species in cases resembling diphtheria when the results of either the clinical or the laboratory examinations are not entirely conclusive.

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A NOTE ON A COLOR CHANGE OBSERVED IN BLOOD CHLORIDE DETERMINATIONS AFTER UPPER GASTROINTESTINAL TRACT OBSTRUCTION*

By Russell L. Haden, M.D., and Thomas G. Orr, M.D., Kansas City, Kansas

IN THE course of some chemical studies on gastrointestinal tract obstruction[1-4] we have made several thousand blood chloride determinations. The first step in the estimation is the addition of 5 c.c. of a standard silver nitrate solution to 10 c.c. of the tungstic acid blood filtrate made by a modification of the Folin-Wu technic described by one of us.[5] In such a determination on a filtrate from normal blood of either man or the dog the precipitated silver chloride usually remains a pure white color even when exposed to direct sunlight. In a preparation made from the blood of a dog with upper gastrointestinal tract obstruction the white precipitate often rapidly changes to a purple, rose, or deep maroon color. The changes are more marked in sunlight. Often, however, the color changes begin immediately after the silver nitrate solution is added showing that sunlight is not necessary for the reaction.

The color changes are not dependent on a variation in chloride content. Changes have been observed in determinations on blood in which little reduction in chloride had taken place as well as in determinations on blood with a very low chloride content. The most marked changes have been observed in determinations on the blood of dogs with obstruction of the cardiac end of the stomach. There is seemingly no direct relation between the level of the toxemia as shown by the nonprotein nitrogen content and the phenomenon described.

To determine what fraction of the blood is responsible for the change, filtrates have been made separately from plasma and cells. Color changes have never been observed in preparations made from filtrates of plasma even when taken from whole blood showing the most marked changes. The filtrates made from the packed cells do show the color changes. Filtrates made from the same corpuscles after washing with physiologic saline solution no longer show the characteristic changes.

Since filtrates from normal blood seldom show this phenomenon, it is evident that some change has taken place subsequent to the gastrointestinal tract obstruction. Several possible explanations may be considered. It is apparent that the color change is due to reduction of a silver compound, the final color being dependent upon the degree of reduction and the extent of dispersion of the silver. It seems possible that after upper gastrointestinal tract obstruction, some body may be present which combines with silver to form an easily reduced compound or is in itself an active reducing body. If such were true very large amounts must be present to account for the extensive color changes and no other evidence of the presence of such a body has been found.

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The change could be accounted for on the basis of photochemical sensitization. Color often develops in the absence of sunlight which would seem to exclude such a possibility. Hematoporphyrin is the only photochemical sensitizer so far known to occur in the animal body. This substance has not been detected in our work.

An aqueous solution of sodium chloride to which the silver nitrate solution is added shows a rapid reduction of the silver chloride. However, if sodium tungstate and sulphuric acid be added to the sodium chloride solution before the addition of the silver nitrate, reduction does not take place even in direct sunlight. In the protein-free filtrate of normal blood made with sodium tungstate and sulphuric acid some element is present which protects the silver chloride against reduction. In the filtrate showing the marked color changes this protection is lost. It seems probable that a loss of inhibition of reduction occurs in upper gastrointestinal tract obstruction.

Certain cross experiments have been made with washed corpuscles and plasma both from normal blood and from blood showing marked color changes. The results have not been entirely uniform. The filtrate from the mixed plasma and washed corpuscles from a positive blood which separately give no reaction, gives the characteristic color changes. The filtrate from washed corpuscles of positive blood mixed with plasma from a normal control also shows color changes. Usually the filtrate from washed corpuscles of control blood mixed with plasma from positive blood shows no color changes in the precipitated silver chloride. These experiments indicate that the color reaction is dependent upon elements in both cells and plasma. The elements in the cells are specific for the change characteristic of upper gastrointestinal tract obstruction. The element supplied by the plasma seems to be nonspecific since normal plasma works equally well.

After blood filtrates have stood overnight in the ice box usually no changes are observed even when the variations in color were most marked in fresh preparations. Precipitates which show marked color changes, after standing a few hours, often return to the normal white color.

The phenomenon described here is of interest as giving possibly some clue to the toxemia characteristic of upper gastrointestinal tract obstruction.

SUMMARY AND CONCLUSIONS

The silver chloride precipitate in chloride determinations in the blood of the dog after upper intestinal tract obstruction is often rapidly reduced with the production of a purple, rose or maroon color.

Such color changes are not observed in chloride determinations on the blood of normal animals.

The color changes do not parallel the rise in nonprotein nitrogen, the chloride level, or the clinical signs of toxemia.

Filtrates made from the plasma show no color changes while filtrates made from the unwashed cells do show such a change.

After washing the cells with physiologic saline solution color changes are no longer apparent unless fresh plasma be added.

The exact cause of the phenomenon is not apparent.

THE NOSTRIL REFLEX IN LUNG DISEASE*

By Hal Bieler, M.D., Pasadena, Calif.

SOME years ago F. M. Pottenger called attention to certain physical signs which attended pulmonary tuberculosis. Atrophy of the skin and subcutaneous tissues, muscle rigidity and atrophy were found to be valuable criteria in estimating the extent of pulmonary disease. Later he observed that the same laws held true in diseases of the abdominal cavity. The sympathetic reflex arc between deep organs and surface structures has been well worked out, and its value as a diagnostic and prognostic aid can scarcely be estimated.

This communication presents evidence which indicates that there is probably a reflex arc between the air-cell capacity of the lungs and the size of the nostrils which Dr. Pottenger thinks is a vagus reflex. A few years ago while attending a case of far advanced pulmonary tuberculosis I noticed that as the end gradually approached the patient's nostrils collapsed more and more. During the last few weeks the left nostril collapsed completely and postmortem showed little or no alveolar tissue present in the left lung. Since that time, every case I have had the opportunity of observing seemed to show a definite relation between nostril size and alveolar cell destruction, always on corresponding sides of the body, but it was not until lately that the time and material at hand permitted a closer and more detailed study.

The first series of cases were studied in the tuberculosis wards at the Los Angeles County Hospital. The nostrils were carefully sketched, Pottenger's signs noted and any additional information that the patient could supply was added. After this series of cases were studied it was decided to examine more cases under better conditions so the work was carried to Olive View Sanatorium at San Fernando, California. There it was not only possible to make very careful bedside examinations with the aid of the house physicians but also to check our nostril findings with the chest films. This was a distinct advantage.

During this study it was also noted that cases of chronic fibroid phthisis, especially the ones that showed clubbing of the fingers and toes also showed a thickening of the nasal tissues surrounding the nostrils.

There are certain cases which at first seem to be exceptions to the rule but a careful observation makes it comparatively easy to differentiate them. These are the cases showing nasal deformity from early rickets. There is not only nasal septum deflection but even deformity of shape and size of the sinuses. The nostrils in these cases at first seem unequal but when carefully measured prove to be the same size, as long as no pulmonary complication exists, although their respective shapes are not identical.

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Fig. 1. Fig. 2. Fig. 3. Fig. 4.

Fig. 1.—Upper: Negro male, aged twenty-two, one month in bed. Muscle spasm and atrophy over left chest. Lower: Negro male, aged seventy-four, sick many years. Slight atrophy and muscle rigidity over right chest.

Fig. 2.—Upper: White male, aged thirty-eight, nine months in bed. Marked atrophy and muscle spasm over left chest. States cavity in left lung. Lower: White male, aged forty, two years in bed. Much atrophy of right chest; right sternomastoid muscle spastic.

Fig. 3.—Upper: White male, aged forty-eight, seven months in bed. Atrophy of left chest; a much depressed supraclavicular fossa; interspaces sunken and muscles rigid. Lower: Negro male, aged fifty-three, three years in bed. Left chest atrophic, painful; left sternomastoid spastic.

Fig. 4.—Upper: Negro male, aged twenty-one, one year in bed. Atrophy above and below left clavicle. Lower: White male, aged twenty-eight, five years in bed. Whole chest markedly atrophic. States large cavity in left lung.

Fig. 5. Fig. 6. Fig. 7. Fig. 8.

Fig. 5.—Upper: Negro male, aged forty-two, two years sick. Right chest shows muscle spasm and atrophy. Lower: White male, aged thirty-one, nine months sick. Apex sunken and marked atrophy on left side.

Fig. 6.—White female, aged twenty-six, five years sick. Dotted lines show approximate shape of nostrils eight years ago. Left pneumothorax, fluid at base.

Fig. 7.—White female, aged forty, eight years sick.

Fig. 8.—White female, aged twenty-four, four years in bed.

The illustrations are self-explanatory, so only a few notes have been appended. Figs. 1 to 5 show the open air ward cases at the Los Angeles General Hospital. Figs. 6 to 19 show cases studied at Olive View Sanatorium. The x-ray findings are given diagramatically; the shading to note degrees of fibrosis; the circles cavities; fluid as dotted areas and normal lung tissue as unshaded. Figs. 20 to 23 show cases of bronchiectasis. It was most interest-

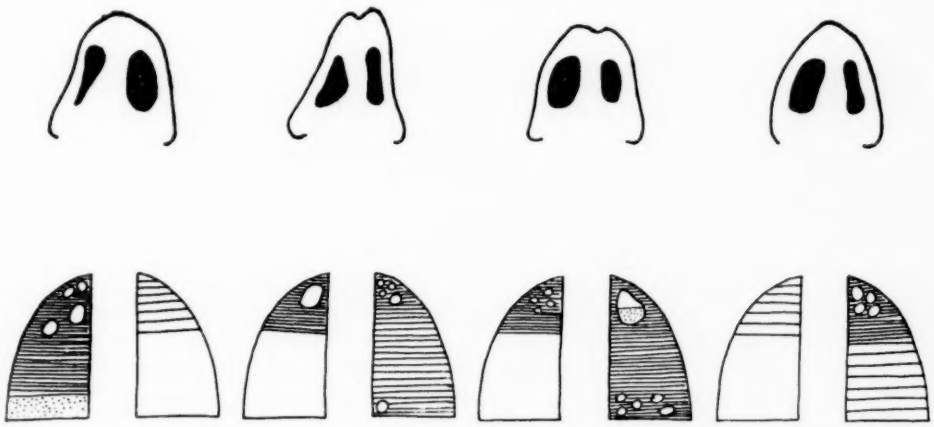

Fig. 9. Fig. 10. Fig. 11. Fig. 12.

Fig. 9.—White female, aged twenty-eight, six years sick.

Fig. 10.—White female, aged thirty, one year in bed.

Fig. 11.—White female, aged forty-one, two years in bed. "Dense nodular infiltration, equally distributed from apex to base. Large apical cavity with fluid level."

Fig. 12.—White female, aged thirty-six, three years in bed. Pneumothorax on left.

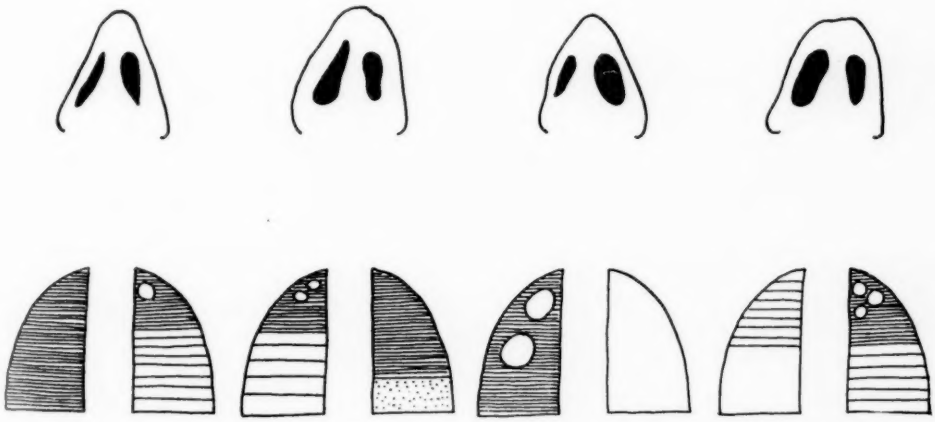

Fig. 13. Fig. 14. Fig. 15. Fig. 16.

Fig. 13.—White female, aged thirty, one year in bed. Right lung shows "very dense fibrosis throughout." Left lung a "dense mottling."

Fig. 14.—White female, aged twenty, ten months in bed. "Heart pushed over to right." Left lung shows "thick walled pleurae; also effusion."

Fig. 15.—White female, aged thirty-five, one and one-half years in bed. Right pneumothorax.

Fig. 16.—White female, aged twenty-eight, one and one-half years in bed.

ing that none of the bronchiectatic cases showed the nostril reflex. Fig. 24 shows a case of actinomycosis of the lung which was kindly offered to me for study by Dr. George Dock of Pasadena. In this case physical examination disclosed that much more of the lung was involved than the x-ray film indicated.

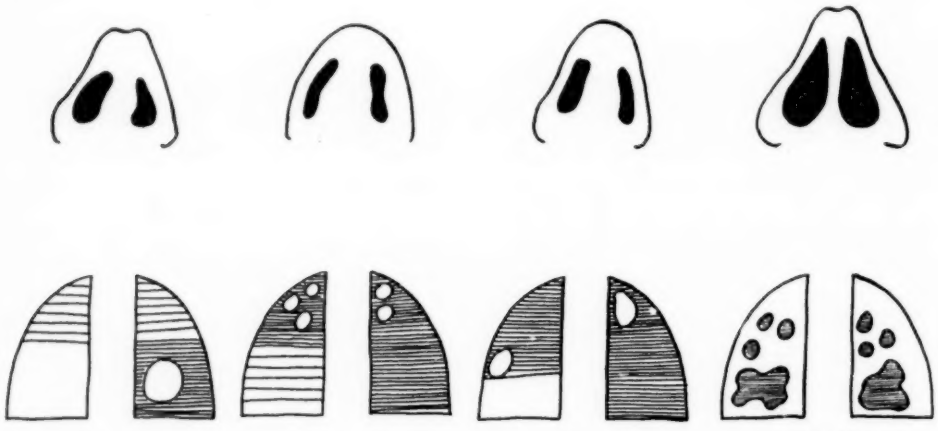

Fig. 17. Fig. 18. Fig. 19. Fig. 20.

Fig. 17.—White female, aged thirty-eight, one and one-half years sick.
Fig. 18.—White female, aged twenty-one, three years in bed.
Fig. 19.—White female, aged twenty-one, three years in bed.
Fig. 20.—White male, aged fifty-two, deceased. Coughed for ten years. Eight attacks of pneumonia (twice double). Copious fetid expectoration.

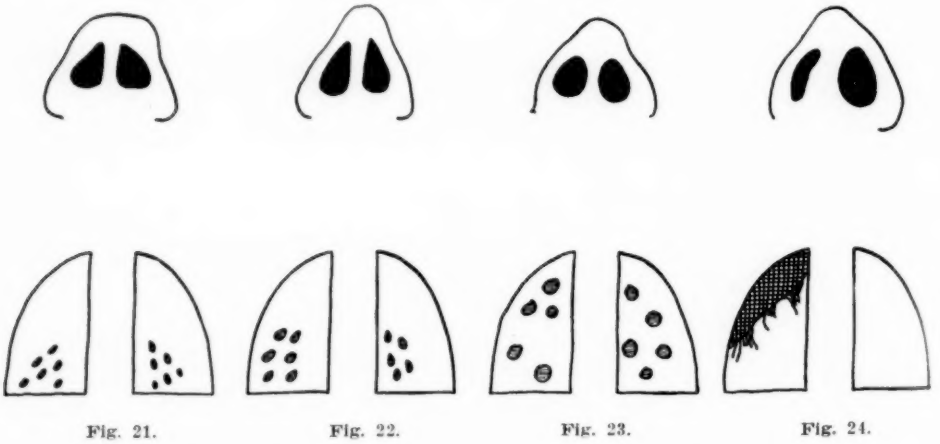

Fig. 21. Fig. 22. Fig. 23. Fig. 24.

Fig. 21.—White female, aged sixteen, coughed three years. Pneumonia twice.
Fig. 22.—White female, aged thirty, coughed five years. Three attacks pneumonia.
Fig. 23.—White female, aged seven, coughed since a baby. Two attacks of pneumonia. One and one-half cups purulent sputum daily.
Fig. 24.—White male, aged 50. Duration probably five years.

CONCLUSIONS

1. The nostril opening bears a relation in size to the alveolar air capacity of the lung on the corresponding side.

2. Probably the reflex does not occur in uncomplicated cases of bronchiectasis. This point may have some importance in differential diagnosis between pulmonary tuberculosis and bronchiectasis.

3. As pulmonary destruction progresses the nostrils, always on sides corresponding to the lungs involved, show atrophy.

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BACTEREMIA DUE TO EBERTHELLA BENTOLENSIS*

With Necropsy Report

By Robert A. Kilduffe,† A.M., M.D., Walter B. Stewart,‡ M.D., and William W. Hersohn, Atlantic City, N. J.

WHILE bacteria in general have long been grouped, in their relation to disease, into pathogenic and nonpathogenic types, it is well recognized that the production of disease as a result of bacterial activities is a phenomenon influenced by a variety of factors involving the virulence and aggressiveness of the bacterium and the resisting powers of the host.

Organisms ordinarily saprophytic may invade the host in such numbers, for example, as to overwhelm the normal mechanism of defense; or, when the invasion is numerically moderate, the virulence may be so high, or the resisting powers of the patient so ineffective that through such or other varied combinations of these as well as other factors, the production of the phenomena characteristic of pathology and disease may follow.

The somewhat loose differentiation of bacteria into pathogens and nonpathogens is, therefore, a flexible division useful as indicating the ordinary ability of bacteria to produce harmful effects.

Exceptions to the rule are of some interest, especially when the organism in question is regarded as essentially a harmless saprophyte, and for this reason the present cases of blood stream infection are reported.

The clinical and necropsy details of the first case in which this organism was etiologically concerned follow:

Case 1.—E. I., male, 40 years old, was admitted to the Atlantic City Hospital, August 11, 1926, on the Medical Service of Drs. E. C. Chew and C. L. Andrews to whom thanks are due for permission to utilize the case for report.

Chief complaint was chills, fever, and vomiting.

History of Present Illness.—Two weeks before admission the patient had attacks of chills and fever, having at least one chill a day. He continued to work, however, and four days before admission consulted a physician who made a diagnosis of malaria and administered

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a large dose of quinine which precipitated attacks of vomiting that continued until his admission to the hospital.

Past Medical History.—The patient has been an actor and professional billiard player keeping very irregular hours, eating irregularly, and working mostly at night. For the past two months he has been an entertainer at a night club.

Fourteen years ago he was in Panama, and since then at irregular intervals has had attacks of chills and fever lasting a day or so and always clearing up promptly upon dietary regulation and intestinal elimination. He has never been free from such attacks for longer than a year and usually not so long.

Physical Examination.—On admission the patient is prostrated and jaundiced with a temperature of 105° F. and a rapid pulse of Corrigan type. The blood pressure was 90/30 in the left, and 90/0 in the right arm.

He presents the physical signs of aortic regurgitation and myocardial weakness. There are no rose-spots, nor is there any enlargement of the spleen. The physical signs in the lungs are not distinctive.

Hb. 80 per cent; R.B.C. 4,130,000; W.B.C. 6,200.

Differential count: Polys. 72 per cent; small mononuclear 23 per cent; large mononuclear 5 per cent. Leucocytic index 2.4.

Repeated examinations for malaria were negative, nor could clinical, bacteriologic, nor serologic evidence of typhoid, paratyphoid, or dysentery infection be found after repeated examinations during the course of the disease.

A blood culture on admission, as well as several succeeding blood cultures, gave a pure growth of a gram-negative, motile bacillus with characteristics to be described below.

The course of the disease was progressive and uninfluenced by treatment, including the intravenous administration of mercurochrome 220-soluble and metaphen. The temperature ranged from 105° to 96°, the high levels being accompanied by chills, the lower by profuse sweating and profound prostration.

The leucocyte count was never over 11,500, with 87 per cent neutrophiles, and more often approximated 6,000, with 78 per cent neutrophiles.

The patient gradually became progressively weaker and more emaciated and extremely anemic. Death occurred September 2, 1926, three weeks after admission.

The report of the autopsy performed a few hours after death may be thus summarized:

The body is that of an adult white male, much emaciated and presenting a well-marked jaundice. There are no external marks of interest.

With the exception of a moderate degree of pulmonary congestion and the presence of palpable glands at the hilus, there are no gross abnormalities of the lungs. There is no evidence of tuberculosis.

The pericardial sac contains about 50 c.c. of blood-stained serous fluid. The heart is not perceptibly enlarged and is covered with a furry, fibrinous exudate. There is an apical adhesion to the pericardial sac. The left ventricle is slightly dilated and the myocardium as a whole is somewhat soft and tears easily.

There is an enormous vegetation (about the size of a walnut) on the aortic valve, and there are numerous small vegetations on the mitral valve which is somewhat thickened.

The stomach and intestines are greatly distended with gas. The liver is slightly enlarged and definitely congested but without evidence of hypertrophy, abscess, or other pathology. The gall bladder is normal.

The spleen is soft, flabby, intensely congested, and about four times the normal size.

The kidneys are grossly normal. The intestines show no lesions of interest except a few small hemorrhagic areas in the lower portion of the ileum.

The cultural characteristics of the organism found in pure culture in the blood on several occasions before death and obtained from the blood, pericardial fluid, peritoneal fluid, liver, and spleen at postmortem were as follows:

Colonies on blood agar and cultures on plain agar slants are colorless or faintly grayish and somewhat moist in appearance, suggesting somewhat those of the Friedlander group but without their viscidity.

The colonies show no tendency to spread or become confluent.

The organism is aerobic and grows best at 37° to 38° C. Gelatin is not liquefied, and a uniform turbidity is produced in broth without the formation of a pellicle. Indol is formed, and acid without coagulation is produced in milk.

The fermentation reactions are shown in the table:

	ACID	GAS
Dextrose	+	0
Levulose	+	0
Galactose	+	0
Maltose	+	0
Lactose	+	0
Sucrose	+	0
Raffinose	+	0
Inisotol	+	0
Mannitol	+	0
Dulcitol	+	0
Salicin	+	0
Glycerol	+	0
Inulin	+	0
Xylose	+	0

Cultures were not agglutinated by B. typhosus, B paratyphosus A., B. paratyphosus B., B. enteritidis, B. coli, or B. dysenteriae agglutinating sera. Unfortunately, the patient's serum was not tested for agglutinins for this organism.

On the basis of the findings above, the organism was identified as Eberthella bentolensis (Castellani and Chambers).

Cultures taken at postmortem resulted as follows:

Pericardial fluid: E. bentolensis and B. coli; right ventricle: E. bentolensis and B. coli; liver: E. bentolensis; spleen: E. bentolensis; peritoneal fluid: E. bentolensis, B. coli, and Staphylococcus aureus; valvular vegetations: E. bentolensis and B. coli.

From the findings above noted there is little reason to doubt the etiologic relationship of E. bentolensis to this patient's illness and death. Whether or not the recurring chills and fever noted in the history were associated with repeated invasions of the blood stream by this organism residing in the vegetations as foci, or from the liver or spleen as a focus, it is, of course, impossible to determine. They remain, however, as logical possibilities.

The second case in which this organism was of apparent etiologic importance occurred on the service of Dr. Walter B. Stewart.

CASE 2.—E. J., female, 8 months old, admitted May 10, 1928, on the tenth day of illness. The attack began with vomiting (once at onset), rhinitis, and cough, dry and unproductive. Since the third day there was an increasing fever ranging from 103° to 105°. There was drowsiness, fretfulness and rapid, moaning respiration.

On admission the physical examination showed a well-nourished child weighing 18 pounds, semi-stuporous with Cheyne-Stokes respiration. Except for the presence of fine, crisp râles at the end of deep inspiration the lungs presented no significant findings.

The leucocyte count was 9,500 with 54 per cent polys and 40 per cent lymphocytes; R.B.C. 3,200,000, Hb. 64 per cent. Lumbar puncture gave a clear fluid without increased pressure and a cell count of 3 per c. mm.

The provisional diagnosis was acute, diffuse bronchopneumonia.

The temperature remained between 103° and 105° until the eighteenth day of the

illness when there was a temporary drop to 101.6°, and when the patient left the hospital on the twenty-first day after her parents signed a release, it was still 102° and 103° and did not finally become normal until the twenty-sixth day of the illness.

By the twelfth day of the illness the respirations had become normal, but peculiar attacks of hyperesthesia were noted, the slightest sound or movement provoking irregular jerkings of the head and extremities after which deep stupor occurred. This was seen only on this day.

At this time the spleen became definitely palpable, the enlargement disappearing within a week.

A radiogram of the chest on admission was reported as follows: In the left chest, about the level of the second rib, there is a small area of increased density, hardly dense enough to be pneumonia. The remainder of the lungs is comparatively clear.

One week later a further report was made: The hilum on both sides shows considerable glandular hyperplasia, and there is an area of density extending from the upper part of the root of the right lung, nodular in character, which is the result of infection of some sort. The appearance of the chest does not suggest miliary tuberculosis.

The stools, at first, were watery, yellow, frequent, and contained mucus.

On the fourteenth day numerous small dull red macules resembling rose-spots in appearance and characteristics appeared on the trunk and buttocks, persisting for two days. The throat at this time showed a small, oval, punched-out ulcer on the left side of the palate.

There was a persistent leucopenia (7,000 to 8,600) with a lymphocyte count from 35 per cent to 50 per cent.

Agglutination tests were negative for B. typhosus, B. paratyphosus, A. and B. paratyphosus B., and feces cultures showed no pathogens.

From the fourteenth to the sixteenth day there was increasing difficulty in swallowing; and at this time signs of meningeal irritation, rigidity of the neck and body, became increasingly prominent, a true opisthotonus occurring on the seventeenth day, with, however, alternating periods of relaxation.

During this period, tremors, hyperesthesia, internal strabismus, and incomplete ptosis of the left eyelid were all noted. Lumbar puncture, however, showed 38 cells per c. mm. as the only abnormality.

At this time a consultant made a diagnosis of disseminated tuberculosis with early meningeal involvement. There was, however, no history of exposure and tuberculin tests were negative.

The true diagnosis was revealed by a blood culture taken on the sixteenth day which showed numerous colonies of a gram-negative bacillus later identified as E. bentolensis. Later in the illness (twentieth day) numerous small pustules appeared on the body from which Staphylococcus aureus was recovered. After a stormy tussle with the furunculosis the patient made a rapid and complete recovery.

The striking feature of the case was the similarity in many respects of the clinical picture to that seen in typhoid fever.

THE NATURE OF THE DEPRESSOR SUBSTANCE IN HEPATIC EXTRACT*

By Alfred Goerner, M.D., and Frank L. Haley, M.D., Brooklyn, N. Y.

SEVERAL years ago our attention was attracted to Major's work[1] on liver depressant substance. With the idea of ascertaining something about the chemical nature of this material, we undertook the following work: A liver extract was prepared as follows: Fresh lamb's liver was passed through a meat chopper and then ground still further with fine sea sand. To this mixture was added physiologic salt solution and toluol as a preservative. The mass was allowed to stand at room temperature for twenty-four hours, and then strained and mixed with an equal volume of 95 per cent alcohol. This alcoholic mixture was filtered through paper after forty-eight hours. To the clear yellow filtrate enough alcohol was added to give a concentration of 75 per cent. After one-half hour the mixture was filtered, giving a clear yellow filtrate. More alcohol was added until a concentration of 80 per cent was obtained. A precipitate formed in the 80 per cent alcoholic extract which was removed by filtration. Some of this precipitate was dissolved in distilled water and on testing gave a slight biuret reaction. The 80 per cent alcoholic filtrate was then made up to an alcoholic concentration of 90 per cent and the precipitate removed by filtration. The following tests were made on this precipitate:

Hopkins-Cole	0	Fehling's	+
Molisch	+	Benedict-Fehling	+
Biuret	0	Chlorides	0
Millon's	0	Uric acid	0
Xanthoproteic	0	Urea	0
Phosphorus	++++	Bile acids	0
Sulphur	+	Bile pigments	0
Cholesterol	0	Choline	0

Some of the 90 per cent filtrate was evaporated to dryness. A brown glistening residue was obtained which was easily soluble in water and gave the following tests:

Lassaigne's method for nitrogen	0
Iron	0
Biuret	0
Millon's	0
Xanthoproteic	0
Hopkins-Cole	0

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It was distinctly positive for Molisch, reduced Fehling's solution well before and after hydrolysis but apparently to a greater extent after hydrolysis. It charred with concentrated sulphuric acid on heating.

The 90 per cent alcoholic precipitate was dissolved in water and enough 95 per cent alcohol added to give a concentration of 50 per cent. No precipitate or turbidity was noticed. An equal volume of acetone was added and the mixture allowed to stand overnight at room temperature. The next morning the supernatant liquid was clear and the bottom of the flask showed a deposit of fine crystals. Some of these were dried at room temperature and then dissolved in 5 c.c. distilled water. One c.c. of this solution injected intravenously into a cat reduced the normal pressure to zero. The animal died within two minutes after being injected. There were no convulsions, just two or three deep respirations.

This precipitate gave a negative test for bile salts but extremely good fusion and ammonium molybdate tests for phosphorus. Quantitative determination of some of the dried crystals showed 51.35 per cent of P_2O_5. Some of the purified crystals showed 98.8 per cent KH_2PO_4.

A second batch of liver extract was then prepared and the acetone precipitate consisting of fine crystals was dried and used in our subsequent experiments. These crystals before purification showed KH_2PO_4 content of 39.17 per cent. Of this precipitate 0.571 gm. corresponding to 0.224 gm. of KH_2PO_4 was dissolved in 1 c.c. water and a cat weighing 3640 gm. was injected with 0.25 c.c. of this solution. The pressure dropped from a normal of 174 mm. to 92 mm. A second injection of 0.25 c.c. was given after the pressure returned to normal and once more the pressure dropped from 174 to 107 mm. A second cat weighing 2050 gm. was then injected with 0.25 c.c. of a solution of pure KH_2PO_4 (0.2 gm. in 1 c.c. of water). The normal pressure dropped from 172 to 92 mm. When the pressure had returned to normal, a second injection of 0.5 c.c. was given and the pressure dropped from 172 to 86 mm.

At this stage of our work it was considered advisable to analyze several liver depressant substances recently placed upon the market by well-known firms designated as "A" and "B." Firm "A" preparation (a solution) showed the presence of approximately 1 per cent KH_2PO_4. Firm "B" preparation gave 2.3 per cent KH_2PO_4. Three solutions representing "A," "B," and our own liver extract were made up to an equivalent strength on a basis of KH_2PO_4 content and all three solutions dialyzed through collodion sacks until we failed to get tests for phosphates in the diffusates. The contents of each sack were then concentrated on a water-bath to an equivalent amount and blood pressure determinations were run on cats with these solutions. None of the solutions showed any depressing effect on the normal pressure.

The respective diffusates were then concentrated on a water-bath to dryness, and the residue dissolved in distilled water. Blood pressure determinations were then made on animals with these solutions and marked depression noted, as shown in our table.

We then used a solution of NaH_2PO_4 equivalent in strength to the potassium phosphate which we had been using in our previous experiments. We found that this solution did not depress the normal blood pressure of cats. It

occurred to us that the potassium ion might be responsible for the drop in pressure. Accordingly solutions of KCl equivalent in strength to the potassium in our phosphate solutions were tried out with a marked reduction in pressure. In the following table will be found a summary of our most important data. Some experiments were run on animals whose pressure had been raised with guanidine solutions previously administered, but the outcome of these was essentially the same.[2] We also observed as did Major[1] that comparatively large doses were necessary to lower the normal pressure of the animals, this being true of our liver extract with a known potassium phosphate content as well as in the case of the pure potassium salt solutions.

GRAPH NO.	BLOOD PRESSURE AT START MM.	DEPRESSION MAXIMUM IN MM.	SOLUTION USED IN C.C.	REMARKS
21	106	106	1 c.c. our own liver extract	Death of animal in 2 min.
22	196	12	0.5 c.c. our extract	
23	196	146	1 c.c. our extract	Death of animal soon after
34	174	82	0.25 c.c. our extract	Pressure returned to normal
35	174	67	0.25 c.c. our extract	Animal survived
36	172	80	0.25 c.c. mono-potass. phosphate	Pressure returned to normal
37	172	86	0.5 c.c. as in No. 36	Pressure returned to normal
45	181	0	1 c.c. of Firm "A" concentrate in sack after dialysis	
46	181	0	1 c.c. of Firm "B" concentrate in sack after dialysis	
47	181	0	1 c.c. of our concentrate in sack	
51	168	32	0.5 c.c. "A" diffusate concentrate	Returned to normal
52	168	25	0.5 c.c. "B" diffusate concentrate	Returned to normal
53	168	36	0.5 c.c. our diffusate concentrate	Returned to normal
62	185	0	0.5 c.c. mono-sodium phosphate	
63	185	0	1 c.c. mono-sodium phosphate	
66	178	21	0.5 c.c. KCl	Returned to normal
67	178	32	1 c.c. KCl	Returned to normal

Animals of nearly the same weight were used in the experiments.

CONCLUSION

As a result of our investigations on liver extract we believe that the depressor substance is monopotassium dihydrogen phosphate with possibly traces of other potassium salts aiding this action. It would also seem that the potassium ion is the active depressor agent since other phosphates fail to cause depression and other potassium salts react positively.

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THE ALLEGED SYNERGISM OF MAGNESIUM SULPHATE AND MORPHINE

Present Status of the Controversy

By Harry Beckman,* M.D., Milwaukee, Wis.

GWATHMEY'S recent discussion of the alleged synergism of magnesium sulphate and morphine[1] provokes this present reply. I shall employ the following topic heads: (1) Definition of Synergism, (2) Prediction of Synergism, (3) Animal Experimentation, (4) Clinical Studies, (5) Summary.

DEFINITION OF SYNERGISM

Synergism is the augmentation of the action of one drug by another, the latter drug to be placed in contact with the organism before or during the application of the drug whose action is augmented. Such a definition has formed the basis of all the pharmacologic investigations of synergism, but Gwathmey, who has been writing on the subject for a good many years, has never accepted it. In his recent paper, just cited, he writes: "By synergism (a Greek derivative meaning 'working together with') is meant the reciprocal augmentation of the action of one drug by that of another." This is exactly what synergism is not. Augmentation takes place, of course, but not necessarily reciprocal augmentation. In my own laboratory, as doubtless in that of any class A medical school, the students each year delight in the spectacular demonstration that magnesium sulphate will synergize urethan, but that the reverse is not true; i.e., urethan will not synergize magnesium sulphate.

PREDICTION OF SYNERGISM

Gwathmey neither in his original pronouncements on the subject of the alleged synergism of magnesium sulphate and morphine[2] nor in the later applications of this synergism which he has proposed[3] has brought forth any animal or human experimental work that proves or even strongly indicates the admissibility of the contention. Therefore, granting that he has at all times remembered that addition and synergism must not be confused the one with the other, it can only be assumed that he still believes this particular synergism must take place because of the essential nature of the substances; i.e., the one (magnesium sulphate) an indifferent narcotic, the other (morphine) an alkaloidal narcotic. That such predictions cannot be made, and that only actual experimental studies can be accepted as having any scientific value, was shown in my review[4] of the literature that appeared during the controversy waged on this point in 1910 to 1915 by Bürgi[5] and his opponents. Space does not permit re-presentation of this mass of conclusive evidence.

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ANIMAL EXPERIMENTATION

I shall discuss here the work of the three investigators who have studied this subject in the laboratory: Issekutz, Gwathmey and Beckman. The studies of Issekutz and myself will be presented only in summary, because of their exhaustive nature; Gwathmey's contribution can easily be presented *in toto*.

(1) *Issekutz.*—In 1915 Issekutz[6] studied the effects of magnesium sulphate on rabbits in the hope of finding some way of increasing its safe range of sedation in the treatment of tetanus. He combined it with other drugs and presented thoroughly convincing experimental evidence of the following: When magnesium sulphate is combined with scopolamine, there is a true potentiation of the action; when it is combined with chloral hydrate, paraldehyde, hedonal, or phenobarbital (luminal), the sum total effect is only addition, and there can be no question of synergism; and when it is combined with morphine, not only does potentiation (synergism) not take place, but also the combination is more toxic than either the magnesium sulphate or the morphine alone.

In 1921 when Gwathmey made his original pronouncement regarding the synergism of magnesium sulphate and morphine,[7] he seems to have been unfamiliar with this work of Issekutz, for he wrote: "A search of the literature does not give a single reference to the use of magnesium sulphate with morphine for analgesic purposes." Nor has he yet taken cognizance of the investigations of the Austrian pharmacologist; though I brought the work to his attention in 1925,[4] and Hatcher reminded him of it again in 1927.[8]

(2) *Gwathmey.*—Gwathmey states that the combination of magnesium sulphate and morphine was first suggested to him by Pellini, and from the work they performed together he offers the following as his sole experimental evidence of the occurrence of the alleged synergism.[7]

"A sufficient number of animal experiments was conducted to prove that one-eighth grain of morphine in 2 c.c. of a 25 per cent solution of chemically pure and sterilized magnesium sulphate, given hypodermically and repeated twice at half-hour intervals, analgized an animal sufficiently for the full force of an artery clamp to be placed anywhere on the skin without being noticed by the subject. The controlled animal, with the same dosage of morphine, given in plain water hypodermically, was not analgized to anything like this degree."

That is all. The size of the animals used is not mentioned, nor is the number of experiments performed stated. Also, to accept the mere statement that dogs given morphine alone were "not analgized to anything like this degree" as one having any scientific value whatever is impossible, especially as it is well known that dogs become markedly insensitive to pinching on even very small doses of morphine; though they will at the same time resist cutting and also show signs of heightened activity of the more superficial reflexes, such as to light and sound. Animal experiments of this sort are utterly without value.

(3) *Beckman.*—Several years ago, while studying ether-oil anesthesia in the laboratory, I undertook to reduce the amount of ether employed by availing myself of the depression induced by the parenteral introduction of mag-

nesium sulphate. Finding very early, however, that the ether was already employed at its minimum effective dose or nearly so, I then directed my attention toward a reduction of the morphine. Here again I employed magnesium sulphate, this time for the synergistic effect that it is alleged to have upon morphine. Early failure to obtain evidence of the occurrence of this synergism, plus the paucity of literature upon the subject led to a thorough investigation. The colonic anesthesia experimental arrangement was well suited to the purpose; an arrangement in which the coincident employment of morphine and ether-oil, in properly adjusted doses, always gave surgical anesthesia. I therefore addressed myself to the task of determining the part played by each factor in bringing about this result, in order that I might definitely fix the amount of sedation accomplished by the morphine. This was necessary if I wished to observe any increase of sedation which might be due to the addition of magnesium sulphate, since *we are unable to describe as synergism the effect induced by the addition of one drug to another unless we have first studied the effect of the original drug alone.* As a result of these initial experiments the amount of sedation always accomplished by the morphine was very definitely determined, as shown in the following brief summary:

(1) When 1 ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 20 pounds (9 kg.) body weight was introduced into the colon of dogs, one hour after the subcutaneous injection of 0.01 gm. morphine sulphate per kilo body weight, safe and satisfactory surgical anesthesia was induced and maintained for a little more than one hour (the criteria for this state of anesthesia, employed in all cases, were described in the reports). (2) When less than the above-stated doses of either ether oil or morphine sulphate or less than the above-stated time interval was employed, anesthesia was not induced even though the other factors were at their effective points. (3) The effective dose of morphine was then fractionated, and it was shown that only beyond 0.0075 gm. per kilo did light anesthesia begin to appear, and that only when the full dose of 0.01 gm. per kilo was employed did the resultant anesthesia satisfy the criteria for the state employed throughout.

Thus it was shown that in a given experimental arrangement a given amount of morphine always accomplished a certain amount of sedation; namely, that amount which was always sufficient to enable the ether to overcome the animal. This was looked upon as establishing the definite sedative power of the morphine under these conditions and was therefore utilized as the basis of subsequent attempts to raise this power by the addition of magnesium sulphate. The drugs were employed as follows in three series of experiments: (a) Effective dose of morphine plus magnesium sulphate, effective time interval, less than effective dose of ether oil. (b) Less than effective dose of morphine plus magnesium sulphate, effective time interval, effective dose of ether oil. The dose of morphine was fractionated here in exactly the same way as in the initial experiments in which the effective dose had been determined. (c) Effective dose of morphine plus magnesium sulphate, but the magnesium sulphate given one-half hour before the morphine, effective time interval, effective dose of ether oil.

In no instance was I able to observe an increase in the sedative power of the morphine due to the employment of magnesium sulphate; i.e., in all cases anesthesia was induced only when all factors were adjusted as had been shown to be necessary in the initial studies in which magnesium sulphate had not been employed. Also a peculiar reaction manifested by the dogs enabled me quite unexpectedly to note that not only was the sedative power of the morphine not raised by the magnesium sulphate but also its analgesic power was likewise unaffected. In the course of the studies 113 experiments were performed, employing 51 animals. Full accounts of the work were published[9] in due detail, together with the conclusion that the alleged synergism does not occur in the dog.

As the result of Gwathmey's attack upon these findings,[10] I sought to clarify the matter by pointing out[11] that issue could be taken with the work only (a) when it could be shown that exactly similar investigations had been made with entirely different results, or (b) when evidence is offered that the subject has been investigated upon animals using an entirely different experimental arrangement, with interpretations at variance with those I made upon my own experimental arrangement. I further stated that, since the alleged synergism of magnesium sulphate and morphine was alone being considered, discussion of the relationship of magnesium sulphate with ether and procaine had nothing to do with the subject and, indeed, served only to confuse the studies.

Gwathmey's reply to this is as follows[1]: "Beckman further states that issue can be taken with his work only when 'it can be shown that exactly similar investigations have been made with entirely different results.' I refuse to do this as his experiments do not disprove the prolonged effect of morphine when magnesium sulphate is used instead of plain water. Further animal experiments are not necessary, as any physician can prove the synergism of magnesium sulphate with morphine by alternating the synergist with water. . . . Beckman states that the synergism of magnesium sulphate with ether and procaine has no bearing on the subject. With this I do not agree. Magnesium sulphate does potentiate the value of many drugs and in different ways. It is reasonable, therefore, to see that since it potentiates other drugs, all such experiments have a bearing on the synergism of the magnesium ion with morphine." His present position, then, so far as I am able to understand it, might be stated as follows: (a) He now throws out all of my work for the reason that it does not deal with the length of time that morphine is assumed to maintain contact with the tissues when it is given with magnesium sulphate, despite the fact that in his own experimental work (previously described in full in this present paper) he concerned himself solely and entirely with the alleged ability of magnesium sulphate to raise the immediate analgesic power of the morphine, and also despite the fact that his previous objections to my work have been put upon the ground that there was a disproportion between my magnesium sulphate and morphine doses. (b) He says that animal experimentation is not necessary anyway, since any practitioner can easily and simply determine whether the effect produced is one of addition or of synergism by injecting a patient with mor-

phine in water and then again in magnesium sulphate solution. (c) He states that this synergism can be assumed to take place because magnesium sulphate synergizes with a number of other substances.

CLINICAL STUDIES

I have several times stated my reasons for believing that none of the published clinical studies of this subject indicate a synergistic effect of magnesium sulphate upon morphine, a position in which I am supported by Hatcher,[8] who in 1927 reviewed the matter for the Council on Pharmacy and Chemistry of the American Medical Association. At this time, therefore, I shall only consider Gwathmey's own clinical proof, of which he has written[10]: "Satisfactory evidence is submitted to show that synergism between magnesium sulphate and morphine *does* occur in man." This evidence is submitted in the form of three tables. Tables II and III comprise lists of cases in which the time before postoperative sedative was shown to be longer when morphine plus magnesium sulphate was used preoperatively than when morphine was used alone. This is certainly no evidence of synergism. Both experimental and clinical literature offer abundant proof that magnesium sulphate is a powerful sedative, which evidence I believe no one has ever sought to question. But nothing is offered in connection with these two tables to show that the increase in sedation was not due to the simple additive effect of the two drugs. Gwathmey's third table, which I present here as Table I, is described by him as "the first classic case in which the potentiation of morphine was established beyond all reasonable doubt."

Having had it pointed out to him[11] that the second longest period of relief

TABLE I

CASE ILLUSTRATING DIFFERENCE BETWEEN MAGNESIUM SULPHATE AND PLAIN WATER WHEN USED WITH MORPHINE (GWATHMEY)

BADLY LACERATED WOUND OF LEG, GAS INFECTION, OPENED WIDE DECEMBER 1; GASTROCNEMIUS SEVERED			
December			Duration Hours
2	1:15 A.M.	Morphine, $\frac{1}{5}$; $MgSO_4$, 2 c.c., 25 per cent; quiet until 5 A.M.	$5\frac{3}{4}$
	6:15 A.M.	Morphine, $\frac{1}{12}$; $MgSO_4$, 2 c.c., 25 per cent; no pain all day	18
		The magnesium was now discontinued. Morphine alone used.	
3	1:30 P.M.	Morphine, $\frac{1}{6}$; pain in two hours	2
	3:25 P.M.	Morphine, $\frac{1}{6}$; pain at 6:40 P.M.	$3\frac{1}{4}$
		The magnesium was again added to the morphine.	
	6:55 P.M.	Morphine, $\frac{1}{6}$; $MgSO_4$, 25 per cent, 2 c.c.; no pain till next day	23
4	5:00 P.M.	Morphine, $\frac{1}{10}$; $MgSO_4$, 2 c.c., 25 per cent; no pain till next A.M.	17
5	10:15 A.M.	Morphine, $\frac{1}{10}$; $MgSO_4$, 3 c.c., 25 per cent; quiet all day	10
	8:15 P.M.	$MgSO_4$, 3 c.c.; very good night	19
6	3:50 P.M.	$MgSO_4$, 3 c.c., 25 per cent; severe pain after three hours	3
	6:15 P.M.	Morphine, $\frac{1}{8}$; $MgSO_4$, 2 c.c., 25 per cent; jerking of leg in two hours	2
	9:15 P.M.	Morphine, $\frac{1}{8}$; $MgSO_4$, 2 c.c., 25 per cent; quiet night	19
		The patient required one hypodermic of morphine, $\frac{1}{10}$ to $\frac{1}{6}$, and $MgSO_4$, 2 c.c., 25 per cent, each day after this until Dec. 13. Codeine and morphine alone did not give relief.	

in this case was experienced in one of the two instances only in which magnesium sulphate was used alone, Gwathmey replies[1]: "Pain was seemingly suppressed for nineteen hours in the same patient by 3 c.c. of magnesium sulphate alone, but this was subsequent to the previous injection of morphine and magnesium sulphate, and one has a right to conclude that a hang-over resulted. This seems to be proved by the fact that with the next hypodermic of magnesium sulphate alone, 3 c.c. of a 25 per cent solution, there was severe pain after three hours, as by this time all the morphine had disappeared from the system." That is to say, the particular effect that followed the 8:15 P.M. injection of magnesium sulphate alone on Dec. 5 is to be attributed to "hang-over" from the previous injection of morphine plus magnesium sulphate, but the good effects that followed the 6:15 A.M. injection on Dec. 2, the 5 P.M. injection on Dec. 4, the 10:15 A.M. injection on Dec. 5, and the 9:15 P.M. injection on Dec. 6 are not to be attributed to hang-over; though they also followed previous injections of the two drugs in combination. These particular effects are to be looked upon as due to synergism!

Furthermore, there is direct evidence in this table that in at least three instances the good results obtained by the combined use of magnesium sulphate and morphine are directly attributable to addition and to addition only—the contention is supported by Gwathmey's own testimony. He has said[1]: "Neither I nor any one else at any time has hinted that 'smaller doses' of morphine could be used 'to overcome the pain' in the presence of magnesium sulphate in any amount. What I said is that 'it seems to act mechanically with morphine, holding it in contact with the tissues longer than morphine can maintain such contact alone.' Hence, as I have distinctly stated, the same amount of morphine must be used with magnesium sulphate as with sterile water. In other words one-eighth grain of morphine is not converted into one-sixth or one-sixth into one-fourth or one-fourth into one-half." Now I understand this statement to mean—and it is the only possible meaning that it can have—that if an inadequate dose of morphine is given to a patient, i.e., a dose much smaller than would be given for the relief of severe pain, then no matter how much magnesium sulphate is given with this inadequate dose the synergistic effect of the magnesium sulphate upon the morphine will not be exhibited, for the reason that the only effect that magnesium sulphate has upon morphine is to hold it for a long time in contact with the tissues, but not in any way to raise either its sedative or analgesic power. Therefore, in the case under consideration, synergism most certainly could not have occurred at the 6:15 A.M. injection on Dec. 2, nor at the 5 P.M. injection on Dec. 4, nor at the 10:15 A.M. injection on Dec. 5, for on all these occasions an entirely inadequate dose of morphine was used. Yet excellent results followed each of these three injections, results which could only have been due to an adequate dose of magnesium sulphate plus a small dose of morphine: that is to say, an additive effect solely and entirely.

SUMMARY

1. Gwathmey does not define synergism in a way that will satisfy any pharmacologist anywhere in the world.

2. Synergism cannot be predicted from the nature of the substances used together, nor can it be presupposed to occur because of analogous synergisms; both which propositions Gwathmey refuses to accept.

3. Issekutz showed that the alleged synergism does not occur in rabbits, and Beckman that it does not occur in dogs. Gwathmey's own experimental work has been shown to be entirely inadequate, but he refuses to perform any experiments in the attempt to overthrow the findings of Issekutz and Beckman; instead, he states that animal experimentation is not necessary, and that any physician so minded can easily prove this synergism for himself.

4. All the clinical work that has been cited by Gwathmey and others has been shown in the reviews of Hatcher and Beckman to prove nothing with regard to the occurrence of this synergism in man.

5. In the laboratory the alleged synergism can be proved to occur only when it can be shown that (a) exactly similar investigations to those of Beckman have been made with entirely different results, or (b) an equally adequate but different experimental arrangement from that used by Beckman has been used with entirely different results.

6. In the clinic the alleged synergism can be proved to occur only when it can be shown that the good result following the coincident use of the two drugs is not due to simple addition of their separate effects.

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COMPARATIVE INHIBITING EFFECT OF GENTIAN VIOLET AND MERCUROCHROME ON THE GROWTH OF CERTAIN FUNGI*

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EARLIER studies[1] on the comparative inhibiting effect of gentian violet and mercurochrome on bacterial growth in vitro elicited some very striking results. It is well established that basic dyes are increasingly effective as inhibiting agents as the P_H of the medium is increased, and that acid dyes show an opposite tendency; i.e., at higher P_H values their effectiveness is less. Gentian violet is a basic dye, while the dye from which mercurochrome is prepared is an acid dye. Thus it was found that at blood P_H gentian violet was exerting a maximal effect while mercurochrome was less effective than in more acid solutions. Table I will illustrate the marked differences in the limiting effective dilutions of the two at P_H 7.4. The results are given for growth after a ninety-six-hour incubation period.

Table I

Organism	Limiting effective dilution		Ratio
	Gentian Violet	Mercurochrome	
B. coli	50,000	1,500	33
B. dysenteriae (Shiga)	200,000	5,000	40
B. diphtheriae	400,000	5,000	80
Staphylococcus aureus	1,000,000	5,000	200

Later it was shown that certain fungi were markedly sensitive to basic dyes,[2] and the question arose whether a similar difference in sensitivity to the above two inhibiting agents would be exhibited by the more complex fungi. In order to determine this, a Blastomyces, two Oidia and three Hyphomycetes were cultured in media containing gentian violet and mercurochrome. The following cultures, used in the experiments described below, were obtained from Dr. M. P. Moon:

Blastomyces
Oidium mycosum (isolated from an infected hand)
Oidium albicans (isolated from an infected tongue)
Trichophyton rosaceum
Trichophyton granulosum
Achorion schoenleinii (Am. Type Culture Collection, H 641)

They were first subcultured in 1 per cent sugar broth and then transferred to nutrient bouillon and agar slants. Since practically all of the cultures used appear to grow readily in a slightly acid nutrient bouillon after repeated transfers, and since they seem to grow readily through only a limited P_H range,

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results are given for growth at only one P_H, which was near the optimum in most cases.

Nutrient bouillon containing varying amounts of gentian violet or mercurochrome and adjusted in reaction by a phosphate mixture in an amount so that the total phosphate concentration was M/15 was inoculated with a loopful of a bouillon culture. In case the fungus grew in mass it was shaken and macerated with a steel needle. Since it was unusually difficult to insure anything like equal amounts of inoculum, an unusually large number of series had to be run and the results homologized, thus differing from the results obtained in studying bacteria, where not only complete series but also gradations in growth within a single series were constantly checked.

Tables II to VII give the results. Data are also included showing the limiting inhibiting dilutions of acid fuchsin. This permits one, by comparison, to note the added inhibiting effect caused by introducing mercury into the acid dye molecule as was done in the case of mercurochrome. The results are given for growth (+) after incubation periods of two, four and eight days respectively.

TABLE II

BLASTOMYCES

GENTIAN VIOLET	P_H		MERCUROCHROME	P_H		ACID FUCHSIN	P_H
DILUTION	6.4	6.8	DILUTION	6.4	6.8	DILUTION	6.4
1 : 500,000	–	–	1 : 5,000	–	–	1 : 25	–
	–	–		–	–		–
	–	–		–	–		–
1 : 1,000,000	–	–	1 : 10,000	–	–	1 : 50	+
	+	–		+	+		+
	+	+		+	+		+
1 : 1,500,000	–?	–	1 : 20,000	+	+	1 : 100	+
	+	+		+	+		+
	+	+		+	+		+

TABLE III

TRICHOPHYTON ROSACEUM

GENTIAN VIOLET	P_H		MERCUROCHROME	P_H		ACID FUCHSIN	P_H
DILUTION	6.4	6.8	DILUTION	6.4	6.8	DILUTION	6.4
1 : 4,000,000	–	–	1 : 5,000	–	–	1 : 25	–
	–	–		–	–		–
	–	–		–	–		–
1 : 8,000,000	–	–	1 : 10,000	–	–	1 : 50	–
	–	–		–	–		–?
	+	–		–	+		+
1 : 9,000,000	+	–	1 : 20,000	+	+	1 : 100	+
	+	+		+	+		+
	+	+		+	+		+

The data in these tables bring out the fact that the higher sensitivity to the basic dye gentian violet on the part of the fungi, compared with that of bacteria under like conditions, is not necessarily explained by assuming that fungi are by nature more sensitive to dyes in general. This is indicated by

TABLE IV

ACHORION SCHOENLEINII

GENTIAN VIOLET DILUTION	P_H 6.4	P_H 6.8	MERCUROCHROME DILUTION	P_H 6.4	P_H 6.8
1 : 8,000,000	–	–	1 : 10,000	–	–
	–	–		–	–
	–	–		–	–
1 : 10,000,000	–	–	1 : 20,000	–	–
	–	–		–	–
	+	–?		–	–
1 : 20,000,000	–?	+	1 : 40,000	–	–
	+	+		–	–
	+	+		+	+
1 : 30,000,000	+	+	1 : 80,000	–	–
	+	+		–	–
	+	+		+	+
			1 : 160,000	+	+
				+	+
				+	+

TABLE V

OIDIUM MYCOSUM

GENTIAN VIOLET DILUTION	P_H 6.4	P_H 6.8	MERCUROCHROME DILUTION	P_H 6.4	P_H 6.8	ACID FUCHSIN DILUTION	P_H 6.4
1 : 10,000,000	–	–	1 : 20,000	–	–	1 : 25	+
	–	–		–	–		+
	–	–		–	–		+
1 : 20,000,000	–	–	1 : 40,000	–?	+	1 : 50	+
	–	–		+	+		+
	+	+		+	+		+
1 : 30,000,000	+	+	1 : 80,000	+	+	1 : 100	+
	+	+		+	+		+
	+	+		+	+		+

TABLE VI

OIDIUM ALBICANS

GENTIAN VIOLET DILUTION	P_H 6.4	P_H 6.8	MERCUROCHROME DILUTION	P_H 6.4	P_H 6.8	ACID FUCHSIN DILUTION	P_H 6.4
1 : 750,000	–	–	1 : 10,000	–	–	1 : 25	–
	–	–		–	–		–
	–	–		–	–		–
1 : 1,000,000	+	–	1 : 20,000	–	–	1 : 50	+
	+	+		–	–		+
	+	+		+	+		+
1 : 1,500,000	+	+	1 : 40,000	–	–	1 : 100	+
	+	+		–	–		+
	+	+		+	+		+
			1 : 80,000	–	+		
				–	+		
				+	+		
			1 : 160,000	+	+		
				+	+		
				+	+		

comparative data contained in Table VIII, which shows that there is no corresponding increase in sensitivity toward the acid dye acid fuchsin. Indeed, if anything, there seems to be a decrease in sensitivity toward this latter dye. Where the acid dye contains mercury, as in the case of mercurochrome, fungi seem somewhat more sensitive to it than are bacteria, and this seems an effect due to the mercury.

TABLE VII

TRICHOPHYTON GRANULOSUM

GENTIAN VIOLET DILUTION	P_H 6.4	P_H 6.8	MERCUROCHROME DILUTION	P_H 6.4	P_H 6.8	ACID FUCHSIN DILUTION	P_H 6.4
1 : 250,000	–	–	1 : 5,000	–	–	1 : 25	+
	–	–		–	–		+
	–	–		–	–		+
1 : 500,000	–	–	1 : 10,000	–	–	1 : 50	+
	+	+		–	+		+
	+	+		+	+		+
1 : 750,000	+	+	1 : 20,000	+	+	1 : 100	+
	+	+		+	+		+
	+	+		+	+		+

Table IX gives a résumé of results. As in the case of bacteria, it is seen that for in vitro work the ordinary basic dye gentian violet is distinctly more effective in inhibiting fungus growth than is mercurochrome. Under the conditions obtaining in the present experiments, the ratio of effectiveness, as shown by limiting dilutions, varies with the organism from 50 to as much as 500 times.

TABLE VIII

ORGANISM	GENTIAN VIOLET DILUTION	MERCUROCHROME DILUTION	ACID FUCHSIN DILUTION
	Bacteria at P_H 6.3		
B. coli (gram-neg.)	1 : 20,000	1 : 2,500	1 : 75
Staphylococcus aureus (gram-pos.)	1 : 2,000,000	1 : 10,000	-----
	Fungi at P_H 6.4		
Blastomyces (gram-neg.)	1 : 500,000	1 : 5,000	1 : 25
Oidium mycosum	1 : 10,000,000	1 : 20,000	1 : <25
Trichophyton rosaceum	1 : 4,000,000	1 : 10,000	1 : 25

TABLE IX

LIMITING DILUTIONS AT P_H 6.4 FOR THE VARIOUS ORGANISMS

ORGANISM	GENTIAN VIOLET DILUTION	MERCUROCHROME DILUTION	RATIO
Oidium mycosum	1 : 10,000,000	1 : 20,000	500
Oidium albicans	1 : 750,000	1 : 10,000	75
Blastomyces	1 : 500,000	1 : 5,000	100
Trichophyton rosaceum	1 : 4,000,000	1 : 10,000	400
Trichophyton granulosum	1 : 250,000	1 : 5,000	50
Achorion schoenleinii	1 : 8,000,000	1 : 20,000	400

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STUDIES OF SEDIMENTATION OF ERYTHROCYTES*

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IN A PREVIOUS article[27] on this subject it was mentioned that although many theories had been advanced with regard to the etiologic factors in sedimentation of blood none had been generally accepted. In view of this I investigated certain cases and attempted to correlate the possible etiologic factors with the clinical data.

That the plasma contains a substance which is responsible for the increased sedimentation of the erythrocytes is the original hypothesis advanced by Fåhraeus. He believed that the increased rate of sedimentation of erythrocytes was due to their increased agglutination, and that this agglutination was chiefly dependent on the properties of the plasma. He concluded that the agglutinating capacity of the plasma was due to the protein fractions, albumin, globulin and fibrinogen. He also observed that solutions of different protein fractions of the plasma differ widely in agglutinating capacity. He said: "Serum albumin aggregates the corpuscles to a very little extent, strong agglutination is produced by the serum globulins. Of the serum globulins, fibrinogen is by far the most active. The strong agglutinating effect of fibrinogen is proved by the fact that the agglutination is to a high degree reduced by defibrination of the blood."

Pfeiffer[43] in his observations on the formation of the "buffy coat" of the blood noted that the increased sedimentation of the corpuscles was generally caused by increased agglutination of the corpuscles. He believed that an increased fibrinogen percentage in the plasma might cause the corpuscles to congregate more rapidly and in this way further the agglutination. Certain other investigators believe that the electrical charge of the erythrocytes exerts a significant influence on sedimentation.

I early observed that by exchanging the cells and plasma of two samples of blood, the cells of the blood in which sedimentation takes place slowly, when transferred to the plasma of the blood in which sedimentation takes place rapidly, gave practically the same result as the original cells in the latter type of blood. If the cells of this blood were transferred to the plasma of the blood that settled slowly, the result was almost the same as when the original cells were in the plasma of this blood (Table I). As this phenomenon occurred quite consistently in all samples of blood obtained from patients who were in compatible groups, as determined by blood typing methods, it would seem that the substance that controls the sedimentation of the erythrocytes is contained in the plasma.

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With Fahraeus' theory in mind, I carried out a number of experiments to determine if possible whether a relationship existed between the fibrinogen content of the blood expressed as fibrin, the sedimentation index and the clinical condition of the patient. All the laboratory and clinical data were tabulated and observed. In other words, my purpose was to correlate any information obtained, either clinically or in the laboratory, with the sedimentation index; I thus hoped to determine the factor or factors that influence the sedimentation of erythrocytes.

The specimens were all obtained from ambulatory patients whose blood was examined as a routine. The method of collecting the specimen of blood and the technic of the sedimentation test were described in my previous article.

TABLE I

MIXTURE OF PLASMA AND CELLS FROM SLOWLY SETTLING AND RAPIDLY SETTLING BLOOD

TIME, HOURS	TUBES A*		TUBES B**		SLOWLY SETTLING BLOOD	RAPIDLY SETTLING BLOOD
0.25	0.050	0.050	0.005	0.002	0.002	0.015
0.50	0.305	0.370	0.012	0.012	0.020	0.220
1.0	0.410	0.440	0.025	0.025	0.060	0.385
2.0	0.465	0.465	0.090	0.085	0.065	0.440
3.0	0.490	0.485	0.012	0.120	0.100	0.460

* Contain 0.5 c.c. of cells from slowly settling blood in 0.5 c.c. plasma from rapidly settling blood.

** Contain 0.5 c.c. cells from rapidly settling blood in 0.5 c.c. plasma from slowly settling blood.

The method used for determining the fibrin content of the blood is one perfected by Schultz, Nicholes and Schaefer.

In the 57 cases in which fibrin determinations were made, there were fourteen men and three women with sedimentation indexes within normal limits (men 0.1 to 2.0 and women 1.1 to 3.4). In this group the average sedi-

TABLE II

NORMAL SEDIMENTATION INDEXES AND NORMAL FIBRIN VALUES IN VARIOUS CONDITIONS

CASE	DIAGNOSIS	AGE AND SEX	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	SEDIMENTATION INDEX	FIBRIN, MG. PER CENT	BLOOD PRESSURE	
							SYSTOLIC	DIASTOLIC
1	Parkinson's syndrome	18M	5.02	82	2.4	266.6	129	80
2	Diabetes mellitus	52M	4.45	75	2.6	257.7	136	84
3	Normal	40M	4.81	73	1.2	169.4	116	80
4	Chronic nervous exhaustion	35M	4.60	84	1.7	193.3	140	75
5	Exophthalmic goiter	19M	4.50	77	1.1	231.1	164	70
6	Migraine	32M	4.80	81	1.3	166.6	125	75
7	Psychoneurosis	36M	4.66	78	2.0	157.7	120	78
8	Asthma	37M	4.29	72	2.0	235.5	116	76
9	Sacroiliac strain	32M	4.75	76	2.1	190.0	120	88
10	Migraine and chronic nervous exhaustion	38M	4.50	78	1.7	190.0	120	90
11	Chronic prostatitis	40M	4.85	79	1.4	108.3	115	85
12	Dementia praecox	27M	4.70	75	0.9	138.6	112	76
13	Duodenal ulcer	25M		77	1.1	195.2	108	72
14	Keloid of chest wall	30M	4.59	76	0.9	115.0	140	74
15	Normal	43F	4.80	81	3.0	245.9	110	75
16	Septic tonsils	47F		73	3.4	182.9	142	88
17	Chronic myocarditis	59F	4.38	71	3.4	266.6	155	85

mentation index for men was 1.6 and the average fibrin values were 178.3 mg. for each 100 c.c. of blood. The average sedimentation index for women was 3.2 and the average fibrin values 231.4 mg. for each 100 c.c. of blood. The average hemoglobin for the men with normal sedimentation index values was 77 per cent on the Dare hemoglobinometer, and the average erythrocyte count was 4,655,000. The average hemoglobin for the women with normal sedimentation index values was 75 per cent on the Dare hemoglobinometer and the average erythrocyte count was 4,590,000. The blood pressure of these patients was normal. None of the women in the group was menstruating.

In all of the cases tabulated in Table II the sedimentation indexes and the fibrin value were normal. Since the normal fibrin value cannot be fixed arbitrarily, I allowed some variation, either above or below the average fibrin values found. The parallelism between the increase in fibrin and the increase in the sedimentation index is shown in Fig. 1.

The sedimentation index was determined on 557 specimens of blood obtained from patients as a routine during their examination and nothing was known concerning them except the name and registration number. In this

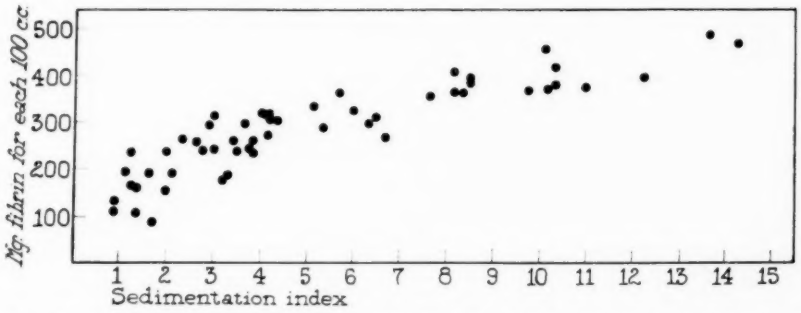

Fig. 1.—The relation between the fibrin content of the blood and the sedimentation index.

group of mixed cases no attempt was made to correlate the results obtained in any one case with another. In fact the histories were not examined for several months after the fibrin and sedimentation index determinations were made.

Gram,[21] in his extensive study of the fibrin content of the blood plasma in health and disease, gives the normal values as: maximal 360, minimal 200, and average 270 for men, and maximal 380, minimal 210, and average 290 for women.

Foster in a series of 42 determinations found the average blood fibrin for normal men to be 163 mg. for each 100 c.c. of blood and for normal women 179 mg. Variations above or below these figures may be said to be within normal limits. The average plasma fibrin for normal men was 332 mg. for each 100 c.c. and for normal women 344 mg. Variations of 50 mg. above or below these figures are probably within normal limits.

That the fibrin content of the blood is increased in a few physiologic conditions and in many pathologic conditions has been established.

PHYSIOLOGIC HYPERINOSIS

Lewinski, Krösing and Landsberg found that the plasma of a pregnant woman is 45, 100, and 20 per cent respectively, richer in fibrin than that of

a nonpregnant woman. De Lee believes that the increase of fibrin is probably a conservative act of nature to prevent dangerous loss of blood during labor. He stated that the amount of fibrin may be increased by one-third.

Fåhraeus found that during pregnancy the "suspension stability" of the blood is almost always greatly reduced; this, according to his theory, means an increase in the fibrin content of the blood.

Gram[22] observed that in the beginning of normal pregnancy there is a tendency to hyperinosis, but that it does not always exceed the upper limit of normal before the fifth or sixth month.

Foster found the average blood fibrin of pregnant women to be 273 mg. for each 100 c.c. and the average plasma fibrin 415 mg. for each 100 c.c. He believes that these figures are probably applicable only for the later months of pregnancy.

Chandler, in a series of duplicate determinations, noted a fairly consistent increase in the fibrin values, particularly in the later months of pregnancy.

Many investigators have studied the changes produced in the blood picture by menstruation, but only a few have investigated the fibrin content of the blood in relation to menstruation.

The fibrin content of the blood has been found to be increased in the newborn and in the aged.

PATHOLOGIC HYPERINOSIS

Fibrin values in pathologic conditions have been subjected to extensive investigation. Although various methods have been devised and used in the determination of fibrin, the results have been fairly constant.

According to Halliburton, Lackschewitz, Berggrün and Dochez, the fibrin has been found to be increased in many infectious diseases, such as pneumonia, pulmonary tuberculosis, pleuritis, peritonitis, acute rheumatic fever, erysipelas and scarlatina.

Pfeiffer[43] was the first to report an extensive series of cases in which the content of fibrin of the blood in disease had been estimated. In general he found an increase of the fibrin of the blood in the presence of diseases which are accompanied by leucocytosis (pneumonia, scarlet fever, erysipelas, and acute rheumatic fever); but in leucemia the fibrin content of the blood was normal. In diseases without leucocytosis (typhoid fever, nephritis and malaria) the content of fibrin was normal.

Kollmann found an increase in the fibrin of the blood of patients suffering from eclampsia; this was not substantiated by Lewinski.

Because of the poor coagulation of leucemic blood it would naturally be supposed that fibrin is deficient. Some investigators have found deficiency in fibrin and others have found an increase. Pfeiffer[44] found the amount of fibrin to be nearly normal. Erben found a normal amount of fibrin in the blood in three cases of myelogenous leucemia and in one case of lymphatic leucemia. According to present knowledge, the failure of leucemic blood to clot cannot be attributed to the decrease in fibrin.

In chlorotic blood, Erben's analysis showed that the total amount of pro-

tein is decreased, chiefly because of the deficiency of hemoglobin; the relation of serum albumin is unchanged, and the proportion of fibrin is increased.

Following severe hemorrhage Drinker and Hurwitz noted a decrease in the fibrin, while in aplastic anemia[28] the amount was normal. In pernicious anemia, according to Wells,[54] the fibrin is decreased in total amount, but is relatively normal as compared with the total proteins. In regard to hemophilia Wells[55] stated: "Since bleeding is normally stopped principally by coagulation a deficiency in fibrin or its antecedents might be expected, but most studies on this point have shown a normal amount of fibrinogen in the blood of hemophiliacs."

The most extensive series of fibrin determinations in health and disease published thus far is that of Gram.[22] His figures for the normal fibrin values have been quoted previously. He found the fibrin content of the plasma to be increased in almost all infectious diseases, in carcinoma, nephritis, pregnancy, polyarthritis, and after injections of sterile milk. A low percentage of fibrin in plasma is found only in certain types of severe degeneration of the liver. Gram found fibrin deficiency in four of a series of 23 cases of pernicious anemia; in the others the fibrin values were within normal limits. In simple anemia the percentage of fibrin in plasma was normal, although the percentage for each 100 c.c. of blood was high. In polycythemia vera the percentage of fibrin in plasma also varied within normal limits, while the percentage of fibrin in blood was low. From the results observed in anemia and polycythemia vera he concluded that the percentage of fibrin in plasma is the value which remains fairly constant when the cell volume is altered. In leucemia, especially in the myeloid type, and in hemophilia he found a slight absolute increase of the fibrin percentage in the plasma.

In a recent study, Foster, in a much smaller series of cases, substantiated Gram's observations.

PREGNANCY

Fåhraeus considered rapid sedimentation of erythrocytes as one of the early signs of pregnancy. Sakae and Tsutsumi also noticed that the rate of sedimentation in pregnant women was far greater than that of normal blood, and that the rate increased as pregnancy advanced. They attributed this to two factors: that the ratio of the blood volume to the erythrocytes in the blood of pregnant women is greater, and that the corpuscles are more easily agglutinated, due to an increase in fibrinogen or globulin or to a decrease in the albumin content of the blood. Gram[21] called attention to the early increase in the sedimentation rate of pregnant women. He believes that the increase in sedimentation in the early months of pregnancy is due to a drop in the percentage of cell volume but in the later months the increased sedimentation rate is carried on by the increase of fibrin. Hirst and Long believe that the sedimentation test is worthless in the diagnosis of early pregnancy. This opinion is also held by Baird and Reis, who examined 100 women and at no time before the twentieth week was the sedimentation time low enough to be distinctive or diagnostic. Falta, after observing the sedimentation reaction in approximately 1000 cases, including patients at various periods of pregnancy,

concluded that the rapidity of sedimentation is constant in pregnancy after the fourth month.

That there is an increase in the fibrin content of the blood as well as an increase in the rate of sedimentation of the erythrocytes of pregnant women seems to be firmly established.

None of my series of 57 specimens of blood in which the fibrin content and sedimentation index were determined was taken from a pregnant woman. In four of the 500 cases in which only the sedimentation indexes were determined, the maximal and minimal sedimentation index was 10.2 and 6.8 respectively.

The sedimentation test as an aid in the diagnosis of pregnancy is of little value in the early months of pregnancy, when other definite signs of pregnancy are lacking. In the later months other signs are so prominent that a more delicate test is not necessary. From the work of other investigators reported in the literature, I am convinced that pregnancy is a physiologic condition in which there is a consistent increase in the sedimentation index, particularly in the later months.

The consensus of opinion seems to be that the sedimentation reaction is of little value in the early diagnosis of pregnancy. This reaction increases with the advance of pregnancy, but is of little use in estimating the termination of gestation.

TUBERCULOSIS

An increase in the sedimentation of erythrocytes in cases of tuberculosis has been noted consistently.

Frisch believes that the breaking down of tissue results in the liberation of enzymes which in turn play an important part in the formation of fibrin. This view is also held by Foster and Whipple, Sakamoto and Mills.

Westergren[56] believes that the fibrinogen fraction of the plasma increases in cases of tuberculosis and that this increase is most easily estimated by means of the sedimentation reaction. In all of his cases which, judging from the clinical signs, he considered active, the extent of the sedimentation of the erythrocytes was parallel to the degree of activity.

Dreyfus and Hecht considered the sedimentation reaction significant in determining the activity of a tuberculous process, and a more reliable indicator of the course of the infection in a given case than the temperature curve.

Morriss, in a preliminary study, observed a definite increase in the velocity of the sedimentation of erythrocytes in active pulmonary tuberculosis. He found also that in the presence of definite symptoms of activity sedimentation was always rapid and in conditions believed to be quiescent the sedimentation was within normal limits, although it was common, especially in women, to find normal readings. His final conclusion was that the test had little value in the diagnosis of tuberculosis. Two years later, in association with Rubin, he substantiated his previous work by saying: "The test has been found especially useful in tuberculosis as an indication of the degree of activity present."

Grafe, Levinson, Salomon and Valtis, Bochner and Wassing, Cooper and many others have found the sedimentation test to be of more or less value in the diagnosis and prognosis of tuberculosis.

Since it has been demonstrated that increased sedimentation of erythrocytes is more active when the fibrin content of the blood is increased and that there is an increase in the fibrin when there is destruction of tissue, especially of the lungs, it seems probable that this is the correct explanation of the increased sedimentation of erythrocytes in cases of tuberculosis.

In summarizing the literature in regard to the use of the sedimentation reaction as an aid in the diagnosis of tuberculosis, the combined conclusion seems to be that the degree of sedimentation depends on, and closely parallels, the extent of anatomic involvement by the disease.

In the series of specimens of blood on which I made fibrin determinations, none was taken in a case diagnosed as tuberculosis. The group of cases in which only the sedimentation index was made is too small to permit conclusions from the results obtained. The blood from all of these patients gave pathologic sedimentation indexes. Three of them were advanced cases of pulmonary tuberculosis with extensive anatomic involvement of the lung. The sedimentation indexes in these three cases were all very high.

Since approximately 17,000 cases of tuberculosis have been studied by this method with similar results, it must be concluded that the value of the test is in the information afforded with regard to the activity and extent of anatomic involvement of the lung. In the early diagnosis of the disease, the sedimentation index is of little value. It might be of some value in helping to eliminate other conditions in which it is known that sedimentation is not increased. After the diagnosis of pulmonary tuberculosis, the test may be of some value in determining whether the patient is growing better or worse. If sedimentation is increased, in the absence of change in the blood count, intercurrent infection, menstruation, or increase in the blood pressure, it would seem to indicate more extensive destruction of lung tissue, with the resulting liberation of more fibrin-forming enzymes. If the reverse of this process is true and the sedimentation index approaches normal limits, it would indicate the possibility of the process becoming less active. In this way the test may be of prognostic value; the higher the sedimentation index the graver the prognosis.

ANEMIA

As previously mentioned, the percentage of fibrin of the blood in simple anemia, aplastic anemia, and polycythemia vera is almost always within normal limits. In leucemia, pseudoleucemia, and hemophilia there is only slight, if any, increase. Diminution of the fibrin percentage of the plasma is usually present in pernicious anemia. Thus some other cause must be attributed to the increase of sedimentation of erythrocytes in cases of anemia, or in some of the blood dyscrasias.

Gram[22] found increased sedimentation of the erythrocytes in anemia that closely parallels the decrease in the blood count. Fibrin values did not increase unless there was secondary infection.

Groedel and Hubert believe that the erythrocyte itself is the most important factor influencing the sedimentation reaction. They applied the test in 304 cases and found that in almost any internal disease, and in normal subjects, the sedimentation rate may be normal or increased. In case of high hemoglobin content and a normal number of erythrocytes the rate is usually

normal, or slightly accelerated. They also noticed that increased sedimentation is more common in blood low in hemoglobin, and the hemoglobin percentage is not related directly either to the two-hour or to the twenty-four-hour sedimentation value.

Westergren[57] has shown, by means of a diagram with plotted curves, that the relation between the sedimentation figures after one hour and after twenty-four hours is chiefly influenced by the hemoglobin content of the blood. By means of this diagram and the sedimentation reading for one hour and twenty-four hours the hemoglobin can be estimated with an error of less than 10 per cent.

Rubin and Smith, in studying the relation between the hemoglobin, cell count, and cell volume and the erythrocyte reaction used hirudinized blood. They found, in general, that their results, as regards the hemoglobin content, agreed with those obtained by Groedel and Hubert. The cell count and sedimentation index were also in agreement. Since the patients in the series had chronic diseases with varying degrees of secondary anemia the decrease in the cell count was accompanied by a proportional decrease in the hemoglobin content.

Erythrocyte counts below 4,000,000 or hemoglobin percentages below 70 are shown in Table III. In this series both the sedimentation index and fibrin

TABLE III

SEDIMENTATION INDEXES AND FIBRIN VALUES ASSOCIATED WITH EITHER LOW ERYTHROCYTE COUNTS OR LOW HEMOGLOBIN PERCENTAGES

CASE	DIAGNOSIS	AGE AND SEX	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	SEDIMENTATION INDEX	FIBRIN, MG. PER CENT
1	Old Pott's fracture	35F	3.89	68	3.8	260.5
*2	Menopause	42F	3.39	60	10.1	454.5
**3	Purpura hemorrhagica; menorrhagia	36F	3.96	57	8.5	393.3
4	Generalized arteriosclerosis	70M	3.96	70	10.3	377.7
5	Fibromyomas	50F	3.31	40	6.4	300.0
6	Secondary anemia (origin not found)	42F	3.17	45	9.7	362.1
7	Diffuse cystitis	57F	3.94	68	6.8	366.6
8	Menorrhagia; secondary anemia	27F	3.94	46	8.5	387.5
9	Generalized tuberculosis	35M	4.27	65	14.4	469.4

*Blood pressure: systolic 156 and diastolic 94.
**Blood pressure: systolic 170 and diastolic 96.

content of the blood were determined. All have increased sedimentation indexes and fibrin values. At first glance it would seem that there is a distinct correlation between increased sedimentation index, increased fibrin and decreased erythrocytes and hemoglobin. More careful examination of the hemoglobin and erythrocytes, with the diagram, will dispel any idea that they can be correlated. In this series there was only one condition in which an increase of fibrin would be expected, and that was in Case 9. In view of the fact that this patient had generalized tuberculosis with extensive pulmonary involvement, it is to be expected that the blood would show an increased fibrin content as well as a high sedimentation index, regardless of the blood picture,

which in this case was normal with the exception of a slight decrease in the hemoglobin percentage.

Cases 3 and 6 are both cases of primary disease of the blood; in Case 6 secondary anemia must be considered potentially a blood dyscrasia since the cause of the anemia could not be found. The hemoglobin and erythrocyte count in both cases are below normal. The sedimentation indexes and the fibrin content of the blood are high. The increased sedimentation index in these

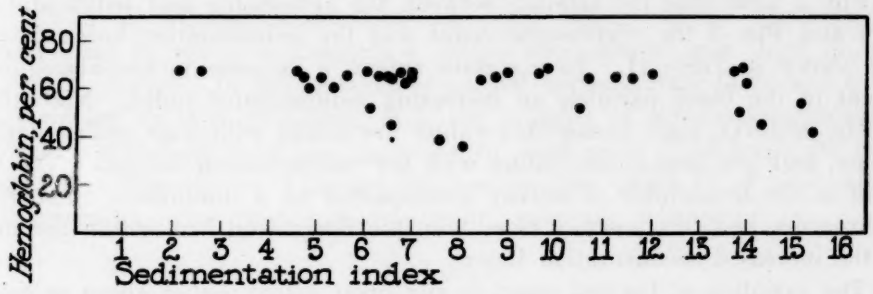

Fig. 2.—The relation between the hemoglobin per cent (Dare) of the blood and the sedimentation index.

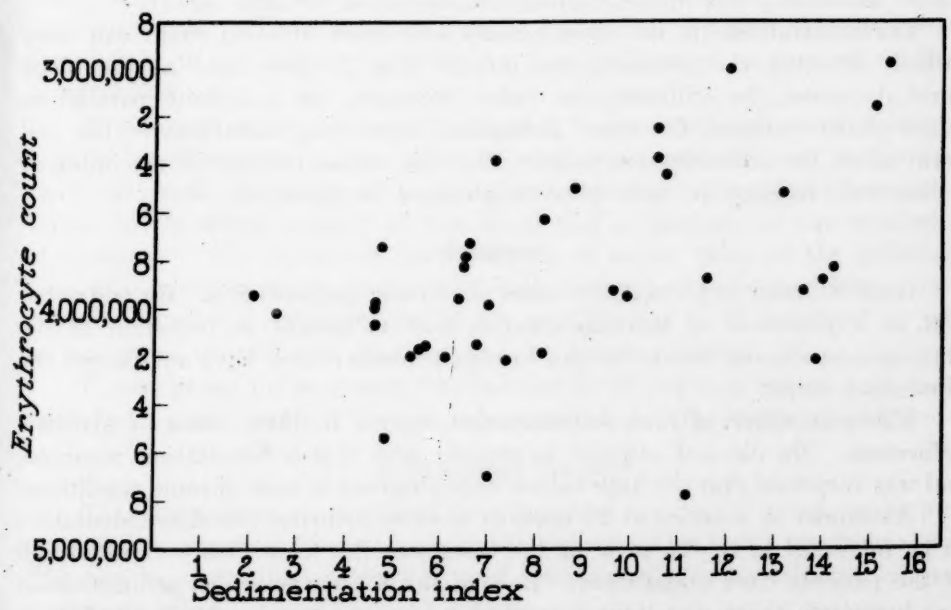

Fig. 3.—The relation between the erythrocyte count and the sedimentation index.

cases can be explained by increased fibrinogen of the blood and by diminished blood count. In Case 4, the erythrocyte count is only slightly diminished, hemoglobin is normal and the fibrin content of 377.7 has a sedimentation index higher than either Case 3 or 6. Cases 1, 2, 5, 8 and 9 show similar variations if compared with each other, or the ones already mentioned. The only correlation that can be made is that in all of the cases the cell count is decreased and the sedimentation index and fibrin are increased. The reason for this

may be that these cases were primarily some other condition associated with abnormal fibrinogen values, with the development later of secondary anemia.

The sedimentation index was determined in 36 cases with maximal hemoglobin percentages of 70 and maximal erythrocyte counts of 4,000,000. Fibrin determinations were not made in this series. As in the previous series, a definite ratio between the erythrocyte count and the sedimentation index could not be obtained. These data agree with those described by Groedel and Hubert, and Rubin and Smith.

Fig. 2 illustrates the relation between the hemoglobin and sedimentation index and Fig. 3 the erythrocyte count and the sedimentation index of the cases shown in Table II. To a certain extent, a decrease in the hemoglobin content of the blood parallels an increasing sedimentation index. Not infrequently, however, high hemoglobin values are found with high sedimentation indexes, and low hemoglobin values with low sedimentation indexes. The decrease in the hemoglobin is usually accompanied by a diminished number of erythrocytes, and this possibly more than the diminished hemoglobin accounts for the increased sedimentation index.

The variation of the cell count to any great extent, either above or below normal limits, affects the sedimentation index. By decreasing the cell volume (which would decrease the cell count) normal sedimentation becomes more rapid. Reversing this process normal sedimentation becomes slower.

The illustrations do not show exactly how much the cell count can vary without affecting the sedimentation index. Fig. 3 shows that as the blood count decreases, the sedimentation index increases, but a definite parallelism is not shown between the two. Influences other than variations of the cell count affect the sedimentation index. For this reason the use of the index as a diagnostic measure in cases of anemia cannot be relied on.

ARTHRITIS

Gram[22] found hyperinosis in cases of chronic polyarthritis. He suggested that an explanation of this phenomenon may be sought in irritation of the fibrinogen-producing organs by the foreign proteins which have not passed the alimentary canal.

Fåhraeus observed high sedimentation figures in three cases of arthritis deformans. He did not attempt to explain why this sedimentation occurred, and was surprised that the high values were obtained in such chronic conditions.

Alexander in a series of 25 cases of chronic arthritis found sedimentation to be increased in 8. In none of the cases was the temperature elevated; all of the patients were ambulatory. In 6 of the 8 cases in which sedimentation was increased, there was later a marked tendency toward acute exacerbation, and during exacerbation the sedimentation rate was accelerated. He concluded that increased sedimentation of the erythrocytes might indicate that the inflammatory process had not entirely subsided, and acute exacerbation might be expected.

Waugh and Nees both stated that the sedimentation reaction was normal in arthritis deformans.

The sedimentation indexes and the fibrin content of the blood were determined in three cases of arthritis. The sedimentation indexes, as well as the

fibrin values, were all above normal. In one case the patient had been menstruating three days. The blood counts and hemoglobin and the blood pressure in all three cases were normal, thus showing that the increase in the sedimentation indexes must be primarily due to hyperinosis.

The sedimentation index was determined in 21 cases diagnosed as arthritis. In about half of this series the sedimentation index was increased. As the blood counts and hemoglobin values were all practically normal, the elevation of the sedimentation index cannot be attributed to the anemia.

From the general examination one could hardly make a distinction between cases in which sedimentation values were normal and those in which they were higher. In the presence of normal sedimentation there was a tendency toward acute exacerbation, as in the presence of abnormal sedimentation indexes. Although there is a tendency in some cases of arthritis for an increase in sedimentation, I do not see how the test could be of any value in this condition either from a diagnostic or prognostic standpoint.

TUMORS

It has never been proved that a malignant growth per se will cause hyperinosis. A tumor obstructing the bile ducts and producing intense icterus may cause distinct hyperinosis although infection and ulceration of the growth is the usual cause. This is substantiated by Foster and Whipple who have shown that injury to any tissue, excepting the liver, will cause an increase of fibrin in the blood.

If a tumor, either benign or malignant, becomes infected, with disintegration and reabsorption of the cell products, two conditions may be produced that will result in acceleration of the sedimentation of erythrocytes: an increase in the fibrin content of the blood and a decrease in the number of erythrocytes. The reports in the literature as to the value of the sedimentation index reaction in the diagnosis of malignant growths are conflicting. Some workers found the sedimentation of the erythrocytes to be increased in cases of malignant growth, and others found the reaction normal.

I determined the sedimentation indexes in 21 cases of benign and malignant tumors and did not observe any specificity of the sedimentation index.

GYNECOLOGIC LESIONS

That hyperinosis is produced by pregnancy and acute suppurative processes with a corresponding increase in the sedimentation index has been shown. The test has been used extensively and satisfactorily as an adjunct in gynecologic diagnosis.

Linzenmeier found it particularly valuable in the differential diagnosis of ectopic gestation and diseases of the adnexa. He stated that as long as the rate increased, virulent bacteria may be present and hence he advises delay in operation.

Haselhorst concluded from the observation of several hundred tests that the sedimentation index reveals the process of inflammation before the temperature or the leucocytes show any change.

Joseph and Marcus, Schumacher and Vogel and Rothe pointed out that

accelerated sedimentation is usually present in association with increased reabsorption of waste products as the result of inflammatory processes.

Friedlaender noted a speeding up of the sedimentation reaction in all infectious processes. He considered the test of little value as an aid in the differential diagnosis of pelvic lesions although it can be used to determine whether a patient with inflammatory adnexal disease, but with a normal temperature and a normal blood count, should be subjected to operation.

Bronnikoff, Falta, Nitschmann and Silzer substantiated Friedlaender's conclusions as regards the infectious process. Falta, Nitschmann and Silzer consider the test of doubtful value in the differential diagnosis of extrauterine pregnancy and tumor of the adnexa.

The use of the test in the differential diagnosis of simple noninfected fibromyomas and a three or four months' fetus when the usual signs of pregnancy were lacking, might be of some value.

The literature seems to show that the test is of practical value in determining the time of operation. During the process of destruction of tissue and cells, hyperinosis occurs; the approximate degree can be determined by the sedimentation index (provided other factors that cause an increased sedimentation index can be eliminated), and so by deductive reasoning the degree of activity of the infectious process can be determined.

In the presence of chronic pelvic infections and in the absence of constitutional signs such as elevation of temperature and leucocytosis, the test is of little value. The sedimentation index may be only slightly, if any, increased, and this may be due to some of the factors mentioned or to a subacute inflammatory process elsewhere.

As a diagnostic aid in pelvic lesions the sedimentation index is not a specific test; it seems to reflect the general condition of the patient. What has been said about the use of the test in gynecologic conditions holds true in general surgical conditions. The degree of sedimentation is not specific in any one group of cases and is to be regarded in the light of a general reaction such as fever and leucocytosis rather than a characteristic test.

DISEASES OF THE THYROID GLAND

Little can be gleaned from the literature on the use of the sedimentation index in diseases of the thyroid gland. Waugh, Alexander and Nees observed that in hypothyroidism and hyperthyroidism sedimentation values were normal.

In the 500 cases in this series in which the sedimentation determinations were made, 46 cases were diseases of the thyroid gland. The sedimentation index varied from low to very high. In the cases in which determinations of fibrin were made a diagnosis of exophthalmic goiter was made in three. In two of these the fibrin values were rather high with correspondingly high sedimentation indexes. The other case was mild exophthalmic goiter, and the determination of fibrin and the sedimentation index were normal.

It is hard to explain the great variations of the sedimentation indexes in diseases of the thyroid gland. Kottmann reported finding a decrease in the fibrin content of the blood. The number of my cases in which determinations of fibrin were made is too small from which to draw conclusions. I did not find a correlation between the sedimentation index and the severity of the dis-

ease. Other factors that influence sedimentation were noticed; for instance, in a few of the cases the cell count decreased. Two patients were pregnant, and two were menstruating. These complicating conditions alone might have caused elevation of the sedimentation index.

Elevation of the sedimentation index in certain diseases of the thyroid gland, especially exophthalmic goiter, is apparent, but without more relation between the sedimentation index and the severity of the symptoms the test could not be of either diagnostic or prognostic value.

HYPERTENSION

While classifying the results obtained from determinations of sedimentation I noted a large group of cases of hypertension in which sedimentation indexes were high. After a more careful scrutiny of the entire series, I also noticed a number of cases of secondary hypertension in which the sedimentation index was high. In the cases in which determinations of fibrin were made I noted four of hypertension; the sedimentation index in all was high.

In explaining the high sedimentation values in these cases, the usual factors, such as hyperinosis or diminished blood count, must be considered.

Gram[22] determined the fibrin in the blood of cases of nephritis with hypertension. In a large percentage of his cases the blood and plasma fibrin were definitely increased. He attributed hyperinosis to secondary infection, predisposed by stasis and edema of the lungs, or to the underlying infection or the toxic effect of organisms in the kidney.

It does not seem possible that in chronic nephritis with hypertension but without pulmonary involvement the infection in the kidney could produce such marked hyperinosis as was found in some of the cases in my series.

Linder, Lundsgaard and Van Slyke, in studying the plasma proteins in nephritis, found that in the presence of nephrosclerosis (Volhard and Fahr classification of nephritis) the plasma proteins were not decreased. In the vascular-interstitial type the effect was the same as in nephrosclerosis but in the glomerulo-tubular or nephrotic type, active or recently active, there was a decrease in the plasma proteins. Fahr and Swanson confirmed the work of these investigators, and also reported an increase of the plasma fibrin in practically all of the cases studied.

I will not attempt to discuss the relationship between nephritis and hypertension. Suffice it to say the relationship between these two conditions has puzzled investigators since the time of Bright. Many theories have been advanced but according to some authorities we are no nearer a solution of the problem than we were fifty years ago.

That hyperinosis is present in the blood of patients with nephritis has been shown. As most patients with hypertension have more or less renal involvement the resulting hyperinosis is expressed by variations of the sedimentation index. Whether or not the sedimentation test is of any value in determining the amount of renal involvement in cases of hypertension remains to be seen.

SUMMARY

A critical study of methods employed in the sedimentation test has been made. By combining and modifying several tests, a simple and expedient test

has been devised. A simple method of reporting results has been proposed, the results being given in terms of a sedimentation index. Average normal values for men are from 0.1 to 2.0 and for women from 1.1 to 3.4, although in occasional instances there are unexplained variations from the average values. Sedimentation tests by this method can readily be compared with those obtained by other methods.

It has been demonstrated that the fibrin content of the plasma, as well as the sedimentation index, is higher in women than in men.

An increase in the plasma fibrin and the sedimentation index is usually present in infancy and early childhood, in old age, during menstruation and during pregnancy.

In pathologic conditions the parallelism between the increase in the fibrin content of the blood plasma and the increase in the sedimentation index of the blood has been shown.

The sedimentation index is of little value in the diagnosis of pregnancy. In the early months the index may not be altered, while in the later months of pregnancy other signs are so marked that the test is not necessary.

The sedimentation index may be of value in determining the degree of activity of a tuberculous process provided cell volume does not diminish. As a primary diagnostic aid there is little to be gained from its use.

Reduction of the cell volume results in an increased sedimentation index, although there is no constant ratio between the results obtained. The use of the sedimentation index as a diagnostic measure in cases of anemia cannot be relied on.

In certain cases of arthritis the sedimentation index is elevated. Variations in either the fibrinogen content or the cell volume may be responsible for this increase. As a diagnostic or prognostic procedure, the test does not have any value.

Either benign or malignant tumor may produce a high sedimentation index depending on the site of the tumor, the presence of infection, the amount of destruction of tissue, or the degree of anemia.

It has not been proved that malignant growths per se will cause an elevation of the sedimentation index. For this reason the test cannot be of use in the differential diagnosis of benign and malignant growths.

As an aid in the differential diagnosis of gynecologic lesions, the sedimentation test has proved to be of some value. It has also been used to determine the time for operating on pelvic lesions of inflammatory origin.

High sedimentation indexes are found in some cases of exophthalmic goiter, and can usually be explained by the anemia present. The test is not of value in determining either the type or degree of hyperthyroidism.

In four cases of hypertension, hyperinosis was noted. With a few exceptions cases of hypertension gave high sedimentation indexes. It is suggested that the degree of nephritis in these cases is responsible for the hyperinosis and the resulting elevation of the sedimentation indexes. It is also suggested that the test may be of value in determining the amount of renal involvement in cases of hypertension.

CONCLUSIONS

1. Variations of the fibrin content of the blood, or of the cell volume (as expressed by the erythrocyte count) exert a marked influence on the sedimentation reaction. It must be admitted that factors other than those mentioned play a part in causing the sedimentation of erythrocytes.

2. During menstruation and pregnancy the fibrin content of the blood increases, and the sedimentation index is higher.

3. The test is known to be of practical value: (a) in determining the degree of activity of a tuberculous process, (b) as an aid in the differential diagnosis of gynecologic lesions, and (c) as an index of the degree of activity present in pelvic infection. In all of the conditions mentioned there is no degree of specificity in the test, and the clinical and laboratory data must be carefully considered.

4. In cases of hypertension sedimentation indexes are usually high. Whether in these cases the use of the test is of value in determining the amount of renal involvement remains to be seen.

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A STUDY OF RETENTION NEPHRITIS*

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THE importance of the determination of inorganic phosphates of blood plasma in nephritis has already been stressed by a number of investigators, among whom may be mentioned Greenwald,[1] Marriott and Howland[2] and Denis and Minot.[3] Schmitz, Rohdenburg and Myers[5] stated that the determination of inorganic phosphates of blood plasma is of definite prognostic value. DeWesselow[4] remarks that nitrogen retention per se is harmless and is only useful because it, to some degree, indicates the degree of kidney damage. On the other hand, he believes that phosphate retention involves certain deleterious results. He observed that phosphate retention is a better guide in regard to immediate prognosis than is urea retention. It draws a sharper line between the fatal and nonfatal cases. In this paper I have attempted to show what importance could be attached to phosphate determinations on a series of nephritic and general hospital cases.

PROCEDURE

The patients studied in the present investigation were largely on the Medical Service of St. Mary's Infirmary. The phosphate determinations were made by the Briggs' modification of the Bell-Doisy method. Prepare a filtrate by adding to one part of oxalated blood plasma three parts of distilled water and one part of 25 per cent trichloracetic acid in an Erlenmeyer flask. Shake mixture, allow it to stand a few minutes, and filter through ashless filter paper. Into a 10 c.c. graduated cylinder deliver 5 c.c. of this plasma filtrate with a 5 c.c. volumetric pipette. To this add 1 c.c. of a 5 per cent ammonium molybdate solution in 5N H_2SO_4 and 1 c.c. of a solution of 5 per cent acid sulphite and 1 per cent hydroquinone. Simultaneously prepare, in another graduated cylinder, a standard made up of 5 c.c. of a phosphate standard (containing 0.03 mg. phosphorus), 1 c.c. of the molybdate and 1 c.c. of the acid sulphite

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hydroquinone solutions. Add distilled water to each of the graduates up to the 10 c.c. mark, invert (with finger over the opening of the graduates) two or three times and allow to stand from fifteen to thirty minutes. Then compare under a DuBoscq colorimeter and calculate the mg. of phosphates in the plasma.[6]

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.03 \times 100 = \text{mg. phosphate per 100 c.c.}$$

The nonprotein nitrogen was determined on a portion of citrated or oxalated whole blood, before centrifuging for phosphate determinations, by the direct nesslerization method of Folin and Wu.[7]

TABLE I

CASES WITH HIGH PHOSPHORUS AND HIGH NPN.

CASE	PHOSPHORUS	NPN.	RATIO	RESULT	DIAGNOSIS OF CAUSE OF DEATH
A– 10	15.0	136.0	1:15.0	D	Uremia
– 71	14.4	254.6	1:17.7	D	Uremia
– 73	14.1	500.0	1:35.4	D	Carc. Pros., Metas. in Lungs. Chr. Neph., Myoc. (A)
– 51	12.0	300.0	1:25.0	D	Uremia
– 15	11.4	150.0	1:13.1	D	Uremia
– 5	11.4	200.0	1:17.5	D	Uremia
B–144	10.0	177.0	1:17.7	D	Chr. Neph., Chr. Myoc.
A– 38	8.0	150.0	1:18.7	D	Chr. Neph., Chr. Myoc., Bronchopneumonia (A)
– 52	7.8	110.0	1:14.1	D	Chr. Neph., Cirrhosis of Liver
– 79	7.1	153.0	1:21.0	D	Chr. Neph., Chr. Myoc.
– 9	5.8	96.0	1:16.5	D	Chr. Diffuse Neph., Chr. Myoc., Arterio (A)
– 8	5.7	140.0	1:24.5	D	Bilat. Congen. Cystic Kidneys (Pneumonperit.)
– 3	5.7	120.0	1:21.1	D	Nephrosis, Chr. Neph.
– 92	5.5	40.0	1: 7.7	D	Chr. Myoc., Aneur. of Aorta, Arterio.
– 20	5.0	92.0	1:18.4	D	Chr. Neph., Chr. Myoc.
–133	5.0	48.4	1: 9.7	D	Chr. Myoc.
– 76	4.7	53.6	1:11.4	D	Chr. Neph., Chr. Myoc.
– 40	4.6	66.8	1:14.5	D	Chr. Myoc.
– 12	4.6	53.1	1:11.5	D	Chr. Neph., Chr. Myoc.
B– 69	4.6	41.4	1: 8.9	D	Suppurative Nephritis (A)
–142	4.3	46.8	1:10.9	D	Chr. Myoc., Auric. Fib.
A– 28	4.1	49.5	1:12.1	D	Chr. Myoc., Chr. Neph., Infarcts of Lungs (A)
– 77	4.1	54.6	1:13.5	D	Thromb. Pulmon. Vess., Encephalomalacia. Arter. (A)
A– 55	6.3	85.7	1:13.6	Unimproved	Chr. Neph., Dental Caries
– 63	5.0	63.2	1:12.6	"	Chr. Neph., Chr. Myoc., Arterio.
B–112	4.8	45.1	1: 9.4	"	Chr. Myoc., Auric. Fib., Asthma, Cirr. Liver
B– 47	5.1	50.0	1: 9.8	Improved	Ac. Bronchitis, Angina Pectoris
– 61	5.0	41.3	1: 8.3	"	Chr. Neph., Intestinal Monilia
A– 59	5.0	40.0	1: 8.0	"	Chr. Neph., Chr. Myoc., Lues
B– 5	4.8	40.0	1: 8.3	"	Chr. Neph.
– 93	4.8	40.0	1: 8.3	"	Early Abortion, Endocervicitis
–132	4.6	42.9	1: 9.3	"	Gen. Arterio., Arterio. Gang., Endart. Oblit.
–100	4.6	40.0	1: 8.7	"	Diab. Mell., Chr. Myoc., Mitral Regurg.
–123	4.5	40.0	1: 9.9	"	Partial Obstruc. Gastro-Enteros. Opening
– 30	4.4	44.4	1:10.0	"	Chr. Myoc.
–130	4.3	40.0	1: 8.3	"	Chr. Neph., Chr. Myoc., Auric. Fib.
– 72	4.2	41.4	1: 9.7	"	Ac. Bronch.
–134	4.3	40.0	1: 9.5	"	Hypertens., Obesity, Enlar. Heart
– 98	4.1	43.0	1:10.4	"	Ac. Rheu. Fever
– 81	4.1	40.0	1: 9.7	"	Simple Colloid Goiter, Chr. Bronch.

A signifies autopsy performed.
Pneumoperit. signifies pneumoperitoneum performed.

For comparison a number of cases were included in the study which had no evidence of nephritis or cardiovascular disease. The average findings on these cases are: phosphate, 3.81 mg., and nonprotein nitrogen, 30.06 mg. A phosphate value above 4 mg. and nonprotein nitrogen of 40 mg. or above were considered high. The results of the study of the various cases are given in the accompanying tables.

RESULTS

The cases have been grouped, according to the laboratory findings, into several classes. Table I is composed of cases with nonprotein nitrogen of 40 mg. or above and phosphate values greater than 4 mg. It is found at once

TABLE II

CASES WITH NORMAL PHOSPHORUS AND HIGH NPN.

CASE	PHOSPHORUS	NPN.	RATIO	RESULT	DIAGNOSIS OF CAUSE OF DEATH
B– 1	4.0	40.0	1:10.0	D	Nephritis
A– 67	3.8	54.5	1:14.3	D	Pneumonconiosis, Chr. Myoc. (A)
– 23	3.7	135.5	1:35.6	D	Chr. Myoc., Lues
– 11	3.7	57.2	1:15.2	D	Ac. Fibrino-Purulent Meningitis (A)
– 43	3.7	40.0	1:10.7	D	Chr. Myoc.
B– 35	3.7	40.7	1:10.9	D	Bulbar Palsy, Gen. Arterio.
A– 30	3.4	44.4	1:12.9	D	Cerb. Hemorr., Chr. Myoc.
B– 38	3.2	80.0	1:24.7	D	Chr. Myoc., Arterio., Chr. Neph. (A)
A– 21	2.2	68.0	1:21.5	D	C. S. Mening., Septicemia, As. Ulcer. Gastro-E.
A– 36	3.2	70.4	1:22.3	D	Lobar Pneu., Arterio., Chr. Neph.
– 48	2.8	46.3	1:16.2	D	Chr. Myoc.
– 32	2.6	50.0	1:19.1	D	Ac. Periton., Perforation of Intes.
– 6	2.5	40.0	1:16.0	D	Lobar Pneu., Chr. Myoc., Chr. Paren. Neph.
– 18	2.5	63.8	1:25.5	D	Hemiplegia, Chr. Myoc. (A)
– 31	2.5	40.0	1:16.0	D	Ulcerative Colitis, Secon. Anemia (A)
– 75	2.3	52.6	1:23.2	D	Pneumonia—Type III, Septicemia (A)
– 72	1.6	52.2	1:33.0	D	Malignancy of Kidneys
B– 83	4.0	50.0	1:12.5	Unimproved	Enlarg. Heart, Chr. Neph.
– 57	4.0	40.0	1:10.0	"	Enlarg. Heart, Hypertens.
A– 33	3.5	42.8	1:12.1	"	Chr. Myoc., Auric. Fib.
– 70	3.2	42,9	1:13.5	"	Lung Abscess
– 60	3.0	61.6	1:20.5	"	Chr. Myoc., Enlar. Heart, Asthma
– 19	2.2	44.4	1:19.9	"	Chr. Myoc., Auric. Fib.
A– 22	4.0	57.0	1:14.2	Improved	Hemiplegia, Lues
B– 71	4.0	40.0	1:10.0	"	Chr. Myoc.
– 57	4.0	40.0	1:10.0	"	Enlar. Heart, Hypertens.
– 95	4.0	40.0	1:10.0	"	Nephrolithiasis
– 48	3.9	41.4	1:10.7	"	Enlar. Heart, Chr. Bronch.
A– 64	3.8	41.8	1:11.0	"	Chr. Myoc., Arterio., Chr. Neph.
– 54	3.6	42.2	1:11.6	"	Cirr. Liver, Ac. Cholecyst.
– 14	3.5	53.3	1:15.1	"	Chr. Myoc., Chr. Neph.
– 2	3.4	40.0	1:11.7	"	Chr. Myoc.
– 20	3.3	43.8	1:13.2	"	Lues (Tert.), G.G. Prostatitis
– 78	3.3	47.7	1:14.7	"	Lues (Tert.)
– 57	3.2	48.0	1:15.2	"	Chr. Bronch., Chir. Neph.
– 58	3.1	40.0	1:12.9	"	Ac. Fibrin. Pleurisy, Ac. Bronch.
– 60	3.0	61.6	1:20.5	"	Chr. Myoc., Enl. Heart, Mit. Regurg., Asth., Chr. Neph.
A– 13	3.0	53.3	1:17.5	Improved	Chr. Myoc., Chr. Neph.
– 37	3.0	42.1	1:14.0	"	Hemoptysis (Cause Undetermined)
– 44	3.0	40.0	1:13.3	"	Fissure in Ano. Fistula in Ano.

A signifies autopsy performed.

that this table includes the vast majority of cases in which nephritic pathology seemed to predominate. Moreover, it is found that of all cases with nonprotein nitrogen greater than 50 and a phosphate value greater than 5, there was not a single case that left the hospital improved. Of the fourteen cases that fall in this group, thirteen died and one left the hospital unimproved. Moreover, in all of these cases a clinical diagnosis of nephritis was made, and in all except one case nephritis was the chief pathology present. In one case in which a prostatic carcinoma was also present the kidneys were found to be very badly damaged at autopsy, and a diagnosis of very severe chronic nephritis was clear. Five cases, all of which had phosphate values greater than eleven, were recorded as having died of uremia. An examination of the ratio of P:NPN in these cases shows that the increase in nonprotein nitrogen is greater than that of the phosphorus. The finding of unfavorable significance is, therefore, a phosphorus value greater than 5 and nonprotein nitrogen greater than 50. Those nephritics, who improved clinically, had in no instance a phosphate retention greater than 5.1.

TABLE III

AVERAGE VALUES

(1) AVERAGE OF CASES WITH NPN. OF 40 MG. OR ABOVE

Average Initial—					*Average Terminal—*			
	No. Cases	*Phos.*	*NPN.*	*Ratio*	*No. Cases*	*Phos.*	*NPN.*	*Ratio*
Died	40	5.4	89.7	1:16.3	15	5.4	94.0	1:17.5
Unimproved	11	4.3	50.2	1:11.7	8	3.9	68.4	1:14.9
Improved	34	4.2	49.8	1:11.7	11	4.0	35.8	1: 9.0

(2) AVERAGE OF CASES WITH NPN. BELOW 40 MG.

Average Initial—					*Average Terminal—*			
	No. Cases	*Phos.*	*NPN.*	*Ratio*	*No. Cases*	*Phos.*	*NPN.*	*Ratio*
Died	22	3.4	33.8	1:10.4	1	2.5	40.0	1:16.0
Unimproved	20	4.1	32.3	1: 7.9	6	4.1	37.5	1: 9.0
Untreated	4	3.5	31.2	1: 8.8				
Improved	84	3.8	32.3	1: 8.6	4	4.0	31.3	1: 7.8

(3) AVERAGE OF CASES WITH RISING NPN. WHO DIED

Average Initial—				*Average Terminal—*			
Cases	*Phos.*	*N.P.N*	*Ratio*	*Phos.*	*NPN.*	*Ratio*	*Cause of Death*
B– 23	7.0	68.0	1: 9.6	3.7	133.5	1:35.6	Nephritis
–144	6.7	46.2	1: 6.9	10.0	177.0	1:17.7	Nephritis
– 38	6.7	66.6	1:10.0	3.2	80.0	1:24.7	Uremia
– 20	6.3	68.0	1:10.8	5.0	92.0	1:18.4	Nephritis
A– 33	6.0	67.5	1:11.2	5.7	120.0	1:21.05	Nephritis
– 71	4.0	90.0	1:22.5	14.4	254.6	1:17.7	Uremia
– 38	3.7	100.0	1:26.7	8.0	150.0	1:18.7	Nephritis
Sums	40.4	506.3	:97.7	50.0	1007.1	1:53.8	
Average	5.7	72.3	1:13.9	7.1	143.8	1:20.9	

(4) AVERAGE OF CASES WITH IMPROVED NPN. WHO DIED

Average Initial—				*Average Terminal—*			
Cases	*Phos.*	*N.P.N*	*Ratio*	*Phos.*	*NPN.*	*Ratio*	*Cause of Death*
A– 52	8.0	150.0	1:18.7	7.8	110.0	1:14.1	Cirrhosis
B– 39	5.0	53.4	1:10.7	3.0	30.0	1:10.0	Cardiac
A– 43	4.3	60.0	1:13.9	3.7	140.0	1:10.7	Cardiac
– 30	2.5	57.2	1:22.9	3.4	44.4	1:12.9	Cerebral Hemorrhage
– 72	1.8	54.6	1:31.0	1.6	52.2	1:33.0	Leucosarc. Kidneys
Sums	21.6	375.2	1:97.2	19.5	276.6	1:80.7	
Average	4.3	75.0	1:19.4	3.9	55.3	1:16.1	

Table II shows cases with a normal phosphate and a high nonprotein nitrogen. In nine fatal cases the nonprotein nitrogen retention was greater than 50 mg. per 100 c.c. In one of those cases a sarcoma of the kidneys was present. In all others an accompanying infection or a serious heart lesion was an important factor in the fatal termination of the illness.

SUMMARY

1. A phosphate retention greater than 5 mg. per 100 c.c. coupled with a nonprotein nitrogen greater than 50 mg. per 100 c.c. indicates severe renal damage.

2. Nonprotein nitrogen values greater than 50, unaccompanied by phosphate retention, are indicative of renal damage, but the immediate prognosis is less grave.

3. Uremic symptoms are most commonly seen in cases with high phosphate retention.

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LABORATORY METHODS

A POTASSIUM FERRICYANIDE METHOD FOR THE DETERMINATION OF REDUCING SUBSTANCES PRESENT IN BLOOD*

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INTRODUCTION

THE determination of the sugar in the blood of invertebrates and cold-blooded vertebrates by a copper method is frequently vitiated by a precipitation of the copper of the oxidizing agent by some substance in the filtrate. To obviate this difficulty the author sought to develop a method which would combine the use of the Folin and Wu[1] blood filtrate and the oxidizing agent of Hagedorn and Jensen.[2]

The principal disadvantages of the Hagedorn and Jensen method are: the relative large error induced by the small amount of blood used and the incomplete precipitation of the blood proteins by zinc, while in many of the copper methods there is fairly rapid reoxidation of the reduced copper. It is thought that the method to be described avoids some of these difficulties.

GENERAL PRINCIPLES

Potassium ferricyanide in a solution made alkaline with sodium carbonate is reduced to potassium ferrocyanide by certain of the reducing substances present in the blood filtrate.

In the presence of an excess of zinc sulphate the potassium ferrocyanide is precipitated. The reaction may be represented by the following equation:

$$2K_4Fe(Cn)_6 + 3ZnSO_4 \rightleftarrows K_2Zn_3[Fe(Cn)_6]_2 + 3K_2SO_4.$$

The potassium zinc ferrocyanide is insoluble in acetic acid and the reaction is completed in one direction.

The unreduced potassium ferricyanide is later reduced by potassium iodide in a solution made acid with acetic acid. The reaction may be represented thus:

$$2K_3Fe(Cn)_6 + 2KI \rightleftarrows 2K_4Fe(Cn)_6 + I_2.$$

The amount of iodine set free is determined by titrating with standard sodium thiosulphate, and so becomes an indirect measure of the reduction brought about by substances in the blood filtrate.

EXPERIMENTAL PART

Precipitation of Proteins.—The blood proteins were precipitated by the technic described by Folin and Wu. An aliquot portion of 5 c.c. is used for the determination.

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Solutions.—All the solutions used were made with Merck's reagent chemicals.

The Alkaline Potassium Ferricyanide Solution was made by dissolving 3.29 gm. of potassium ferricyanide and 85.5 gm. of anhydrous sodium carbonate in distilled water and diluting to the final volume of one liter.

G. Kassner[3] reports that potassium ferricyanide solutions of alkaline earths are unstable. Sodium hydroxide causes it to deteriorate rapidly. The rate of this change of alkaline potassium ferricyanide solution is indicated in Table I. Light is an important factor in affecting the rate of change.

A 5 Per Cent Zinc Sulphate Solution was made with the salt which contained seven molecules of water of crystallization.

A 10 Per Cent Potassium Iodide Solution. This solution should be tested with starch and discarded if free iodine is found.

TABLE I

RATE OF CHANGE OF THE ALKALINE POTASSIUM FERRICYANIDE ON STANDING EXPOSED TO DAYLIGHT

NO. OF DAYS	NO. OF DETERM.	BLANK DETERM. N/100 C.C. THIO.	MEAN DEVIATION	PERCENTAGE OF CHANGE
		In Colorless Glass Bottle		
0	8	10.00	0.008	0.0
1	7	10.00	0.009	0.0
4	10	9.98	0.041	0.2
6	5	9.96	0.022	0.4
15	8	9.94	0.026	0.6
34	10	9.62	0.033	3.8
67	11	8.95	0.053	10.5
		In Amber-Colored Bottle		
0	4	10.02	0.030	0.0
98	5	10.02	0.023	0.0

Determination.—General Discussion: Quantitative care should be exercised in making the protein-free blood filtrate, and the N/100 sodium thiosulphate; in pipetting the filtrate and the alkaline potassium ferricyanide solutions; and in the final titration. The making and pipetting of the potassium iodide, zinc sulphate and acetic acid solutions need not be so carefully done. Changes in temperature of the boiling water-bath during the reduction reaction due to ordinary changes in atmospheric pressure produced no noticeable change in the final titration.

The zinc sulphate solution should be so delivered into the alkaline potassium ferricyanide that its rapid and uniform distribution is assured and precipitation of the potassium ferricyanide by the zinc is minimized. This may be accomplished by forcibly blowing the zinc sulphate solution from a pipette.

Technic: An aliquot portion of 5 c.c. of protein-free blood filtrate is carefully pipetted into a test tube of about 20 mm. x 170 mm. Ten c.c. of the alkaline potassium ferricyanide solution are added and the test tube is placed in boiling water for fifteen minutes. It is removed and then cooled at once by placing it in cold running water. After the solution is cooled, the determination may be continued at any convenient time within three hours, see Table II.

Just before titrating, 5 c.c. of zinc sulphate solution are added, then 2

c.c. of potassium ferricyanide solution and 5 c.c. of approximately 30 per cent acetic acid. The titration with N/100 sodium thiosulphate should be completed within the next minute or two. Two or 3 drops of 1 per cent starch solution are added immediately before the color due to the free iodine disappears. The end-point is the disappearance of the blue color.

The blank determination may be made in the following manner: a solution may be made which contains 1 volume of 10 per cent sodium tungstate, 1 volume of ⅔ N sulphuric acid, and 8 volumes of water. Five c.c. of this solution replaces 5 c.c. of the protein-free blood filtrate in the technic described above. Five c.c. of distilled water may also be used to replace the blood filtrate.

TABLE II

A COMPARISON OF THE EFFECT OF TIME OF STANDING ON THE AMOUNT OF REDUCTION OF POTASSIUM FERRICYANIDE SOLUTION BY GLUCOSE AT ROOM TEMPERATURE OF APPROXIMATELY 18° C.

TIME IN HOURS	GLUCOSE ADDED MG.	NO. OF TITRATIONS	N/100 THIO. C.C.	MEAN DEVIATION
0.0	1.50	12	4.54	0.032
1.0	1.50	6	4.55	0.041
2.0	1.50	6	4.55	0.027
3.0	1.50	16	4.58	0.036

TABLE III

A COMPARISON OF THE EFFECT OF THE MAXIMUM AMOUNT OF SODIUM TUNGSTATE, 5 C.C. OF FOLIN AND WU FILTRATE CONTAINING THE INDICATED AMOUNT OF STANDARD GLUCOSE, AND OF NO SODIUM TUNGSTATE, 5 C.C. OF WATER ALSO CONTAINING THE INDICATED AMOUNT OF STANDARD GLUCOSE, ON THE REDUCTION OF 10.0 C.C. OF N/200 ALKALINE POTASSIUM FERRICYANIDE

NUMBER OF TITRATIONS	C.C. N/100 THIO.	ACTUAL GLUCOSE ADDED MG.	GLUCOSE PER 100 C.C. MG.	MEAN DEVIATION	MEAN DEVIATION OF MEAN
			Filtrate		
16	1.58	0.50	50	±0.058	±0.015
14	2.37	0.75	75	±0.054	±0.015
14	3.15	1.00	100	±0.028	±0.008
15	4.58	1.50	150	±0.043	±0.011
15	6.11	2.00	200	±0.042	±0.011
28	7.54	2.50	250	±0.038	±0.007
21	8.96	3.00	300	±0.054	±0.012
			Water		
43	0.32	0.10	10	±0.042	±0.0064
34	0.86	0.25	25	±0.047	±0.0081
17	1.55	0.50	50	±0.035	±0.0085
12	2.32	0.75	75	±0.030	±0.0092
16	3.02	1.00	100	±0.037	±0.0092
12	4.54	1.50	150	±0.032	±0.0092
12	5.99	2.00	200	±0.034	±0.0099
27	7.42	2.50	250	±0.034	±0.0065
19	8.79	3.00	300	±0.002	±0.0004

Calculation.—The amount of N/100 sodium thiosulphate required by the free iodine in the unknown is subtracted from the amount required by the blank. The difference is proportional to the amount of potassium ferricyanide that has been reduced by the reducing substances in the blood filtrate. Since the aliquot represents but 0.5 c.c. of blood, the result is multiplied by 2

and by 100 to obtain the reducing power per 100 c.c. of blood. In Tables III and IV this has been done.

Glucose Standard: Table III is based upon results obtained from a sample of glucose furnished by the United States Bureau of Standards. The specific rotation was stated to be as follows:

$$[\alpha]\ \frac{20.0}{5461A} = 62.032 + 0.0422p + 0.0001897p^2$$

Where p is the percentage concentration. Glucose Sample 2, prepared by Duggan and Scott[4] agreed with the above in its reducing power.

A carefully weighed amount of glucose was dissolved in a suitable volume

TABLE IV

AMOUNTS OF GLUCOSE AND GLUCOSE CONCENTRATION OF BLOOD CORRESPONDING TO VARYING AMOUNTS OF N/100 SODIUM THIOSULPHATE

N/100 THIOSULPHATE C.C.	ACTUAL GLUCOSE INDICATED MG.	GLUCOSE IN 100 C.C. OF BLOOD, MG.	N/100 THIOSULPHATE C.C.	ACTUAL GLUCOSE INDICATED MG.	GLUCOSE IN 100 C.C. OF BLOOD, MG.
0.10	0.03	6	4.50	1.49	298
0.20	0.06	12	4.60	1.52	304
0.30	0.09	18	4.70	1.55	310
0.40	0.12	24	4.80	1.59	318
0.50	0.15	30	4.90	1.63	326
0.60	0.18	36	5.00	1.66	332
0.70	0.21	42	5.10	1.69	338
0.80	0.23	46	5.20	1.73	346
0.90	0.27	54	5.30	1.76	352
1.00	0.30	60	5.40	1.80	360
1.10	0.34	68	5.50	1.83	366
1.20	0.37	74	5.60	1.86	372
1.30	0.41	82	5.70	1.90	380
1.40	0.45	90	5.80	1.93	386
1.50	0.48	96	5.90	1.97	394
1.60	0.52	104	6.00	2.00	400
1.70	0.55	110	6.10	2.04	408
1.80	0.58	116	6.20	2.07	414
1.90	0.61	122	6.30	2.11	422
2.00	0.65	130	6.40	2.14	428
2.10	0.68	136	6.50	2.18	436
2.20	0.71	142	6.60	2.21	442
2.30	0.74	148	6.70	2.25	450
2.40	0.78	156	6.80	2.28	456
2.50	0.81	162	6.90	2.32	464
2.60	0.85	170	7.00	2.35	470
2.70	0.89	178	7.10	2.39	478
2.80	0.92	184	7.20	2.42	484
2.90	0.95	190	7.30	2.46	492
3.00	0.98	196	7.40	2.49	498
3.10	1.03	206	7.50	2.53	506
3.20	1.06	212	7.60	2.57	514
3.30	1.09	218	7.70	2.60	520
3.40	1.13	226	7.80	2.64	528
3.50	1.16	232	7.90	2.68	536
3.60	1.19	238	8.00	2.71	542
3.70	1.22	244	8.10	2.75	550
3.80	1.26	252	8.20	2.79	558
3.90	1.29	258	8.30	2.82	564
4.00	1.32	264	8.40	2.86	572
4.10	1.35	270	8.50	2.89	578
4.20	1.39	278	8.60	2.93	586
4.30	1.42	284	8.70	2.97	594
4.40	1.45	290	8.80	3.00	600

of water. Twenty c.c. of this solution were diluted to 100 c.c. Five c.c. of the latter, corresponding to 1 c.c. of the original solution, were used for each determination.

Results of the Work With Blood.—A few experiments were carried out with the Shaffer-Hartmann method and my method simultaneously in order to compare the oxidizing power of the two methods on the protein-free blood filtrate of the blood of different animals. Table V shows that my method constantly gives higher values for the amount of reducing substances present in the filtrate. There is no apparent stoicheiometrical relationship between the two methods.

TABLE V

A COMPARISON OF THE SHAFFER-HARTMANN AND THE AUTHOR'S METHOD ON THE AMOUNT OF REDUCING SUBSTANCES IN THE PROTEIN-FREE FILTRATE OF THE BLOOD OF DIFFERENT ANIMALS

BLOOD	MG. OF GLUCOSE PER 100 C.C. OF BLOOD						DIFFERENCE
	SHAFFER-HARTMANN			AUTHOR			
	NO. OF DETERM.			NO. OF DETERM.			
Cat (etherized)	(7)	274	±5.4	(6)	311	±2.9	37
Ox (fresh)	(4)	79	±1.0	(4)	121	±1.5	43
Ox (fermented)	(5)	24	±1.9	(5)	44	±1.5	20
Rabbit (fresh)	(10)	143	±2.7	(10)	166	±5.0	23
Alligator (fresh)	(6)	69	±3.5	(4)	112	±1.5	43

From the above results it appears that other substances than glucose are oxidized by the potassium ferricyanide. While this is disappointing in some measure, the difficulty is probably one of degree only for it is generally conceded that none of the methods in general use, except possibly the latest methods of Benedict[5] and of Folin,[6] are strictly specific for reducing sugars.

The method is useful in spite of its lack of specificity for measuring changes in the concentration of the reducing substances, especially so when these changes are presumably due solely to changes in the concentration of glucose as in insulin studies or work involving the feeding or the injection of glucose. Its use, in place of one of the copper methods, is to be preferred in studying the blood of certain invertebrates and of cold-blooded vertebrates.

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THE BENZIDINE REACTION*

Some Observations Relating to Its Clinical Application

By Robert G. Bramkamp, A.B., Banning, Calif.

THESE experiments were undertaken to determine the quantity of blood introduced into the alimentary tract necessary to give a positive benzidine reaction in the stool. The relation of the test to the diet was also considered.

The benzidine test is simple of application, and is quite generally used in clinical practice. Its sensitiveness for blood arising from different parts of the alimentary tract is therefore of practical importance.

The only work relating to the quantity of ingested blood required to give a positive stool reaction is that of Abrahams,[1] who, after living on a hemoglobin and chlorophyll-free diet, found that 1 c.c. gave a positive reaction. His experiments were apparently not continued.

Ogilvie[2] states that any exclusion of meats from the diet (except for large quantities of rare meat) is unnecessary. Other than these experiments there has been little done relating directly to the effect of diet upon the test for occult blood, although many of those who have worked with the test recommend that a meat-free diet be instituted several days before the stool is to be examined.

EXPERIMENTAL

I. *Technic.*—Three general methods were used:

1. (For standardizing the reagents.) Two c.c. of the solution to be tested are mixed with 1 c.c. of half saturated benzidine in alcohol. Lyle, Curtman, and Marshall[3] advise against the use of alcoholic solutions, but with fresh preparations quite satisfactory blanks are obtained. To this mixture are added 0.5 c.c. of glacial acetic acid and 0.3 c.c. of hydrogen peroxide (3 per cent). A blue zone at the line of junction of the peroxide with the other solution indicates the presence of blood. This test is not applied to fecal material. For the latter, the following were used:

2. (Recommended by O. & R. Adler.) After preliminary ether extraction the stool is treated with about one-third its volume of acetic acid and thoroughly mixed. The mixture is then extracted with another portion of ether. The ethereal extract is mixed with 1 c.c. of benzidine solution, and 0.3 c.c. of hydrogen peroxide is added. A positive reaction is evidenced, by the appearance of a blue color at the line between the last two liquids.

3. The slide test of Wagner. A smear of the stool is made on a slide and heated for a short time. Upon the smear are placed two drops of acetic acid, followed by eight drops of benzidine solution and two of hydrogen peroxide. In the presence of blood the smear and liquid become bright blue.

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II. *Standardization of Reagents.*—Using diluted hemolyzed blood and the first technic, blood was detected in a dilution of 1 to 25,000. This does not approach the sensitiveness attained by some, but we wished to have a technic within the practical working range of the test so that the results would be more applicable to clinical work. It is possible to refine the technic until ordinary tap water will give a positive reaction.

The ether extraction method applied to hemolyzed blood, detected blood in a dilution of 1 to 10,000. The method is thus about 4/10 as sensitive as the preceding one.

III. The next step was to determine the quantity of fresh blood mixed with feces which would give a positive test. For this, tests 2 and 3 were used. To obtain fecal material for the test the subject ingested only foods which, when ready to eat, gave a negative reaction to the benzidine test.

RELATIVE SENSITIVENESS OF THE TESTS

	1	2	3
	1 cc. BENZIDINE SOLN. 0.3 cc. ACETIC ACID 0.3 cc. H_2O_2	BLOOD ADDED TO 2 cc H_2O; ½ VOLUME OF HAc ADDED; EXTRACTED WITH 5 cc. Et_2O. TEST AS IN 1 APPLIED TO ETHEREAL EXTRACT.	TEST AS IN 2 EXCEPT THAT 2 GM. FECES WERE SUBSTITUTED FOR THE 2 cc. H_2O USED IN 2.
GRAMS BLOOD	REACTION	TO THE	TEST
0.00004	—	—	—
0.00008	+	—	—
0.00016	+	+	—
0.0008	+	+	+

Chart 1.

Such foods are well-cooked potatoes, milk (some samples gave a slight positive reaction), cheese, most prepared breakfast foods, butter, and many fruits. All cooked and preserved meats that were tested gave positive reactions. The feces obtained on this diet were uniformly negative to the test.

Hemolyzed blood was mixed with the feces. Two grams of feces were used instead of the 2 c.c. of solution employed in the previous ether extraction test. As is shown in Chart 1 the sensitiveness of the ether extraction test was reduced to one-fifth that obtained using water instead of feces. On the basis of this fresh blood test and assuming the weight of a stool to be 200 gm., it would appear that as small an amount as 0.08 c.c. of blood in the stool is theoretically detectable.

IV. *Quantity of Ingested Blood Necessary to Give a Reaction.*—In determining the reaction to ingested blood the test-negative diet was used, containing no substance which would give a positive reaction when ready to eat. The stools were marked by the subject ingesting a capsule containing 100 mg. of carmine at the beginning of the test period. The blood (taken from the vein of the subject) was diluted with water and taken after the noon meal,

the carmine marker having been taken about three hours before. The slide and ether extraction tests were both applied to the feces. They agreed in practically all cases. The ingestion of 3.5 gm. of blood (corresponding to 3.6 per cent of the weight of the feces obtained) gave a positive reaction; 3.0 gm. gave negative results. Two gm. of dried blood in a gelatin capsule produced a positive reaction. This dried blood might be considered as analogous to clotted blood forming after hemorrhage. The results are shown in Chart 2. It is seen that about ninety times as much blood is needed to give a positive reaction when taken by mouth as is required when the blood is mixed fresh with the feces. To determine where this change occurs the following tests were carried out:

V. *a. Gastric Digestion.*—A sample of gastric juice was withdrawn one hour after drinking 250 c.c. of water. Analysis showed:

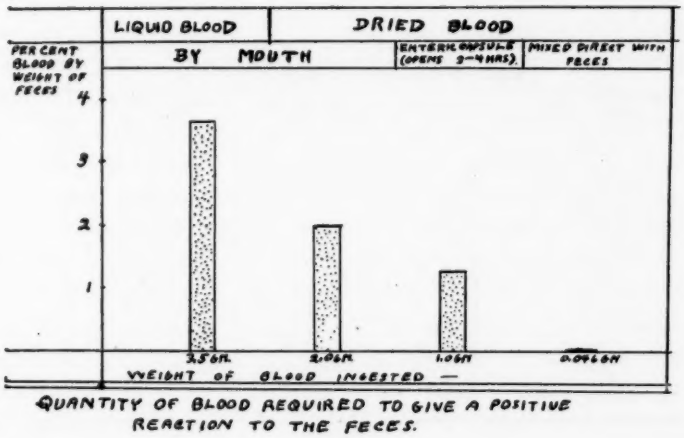

Chart 2.

Total acidity	36.0
Total free acidity	26.0
Free HCl	21.0
Peptic activity	48.0

To 10 c.c. of the juice 40 c.mm. of blood were added. After digesting for twenty-four hours the mixture was diluted, and it was found, using method 1, that blood was still detectable in a dilution of 1/25,000 of the original quantity. It is probable, therefore, that gastric digestion does not materially lessen the reacting power of the blood.

b. Pancreatic Digestion.—One gram of pancreatin was mixed with 10 c.c. of 1/20 blood in 0.5 per cent sodium carbonate solution. The proteolytic activity of the mixture was evidenced by the digestion of 1 mm. of egg white in a Mett tube within four hours. After twenty-four hours, dilution of the solution showed that it required 0.2 c.c. of a 1/1250 dilution to give a positive reaction—a detection of the blood in a dilution of 1/12500. The reacting blood had been reduced by the pancreatic digestion to about one-half the amount present in the original mixture. It is evident, then, that digestion in

the intestine is important in decomposing the blood to products which do not react to the benzidine test. Dialysis of the digestion mixtures gave no indication that any of the reacting substances could pass through a semipermeable membrane.

c. Bacterial Decomposition.—Two c.c. of 50 per cent blood in water were inoculated with three loopfuls of a heavy fecal suspension. After incubating for forty-eight hours the solution was diluted to 1/1250 of the original blood volume, and it was found that 0.1 c.c. of the solution just gave a positive reaction; therefore, there had been no reduction of the reacting blood by bacterial decomposition such as would occur in the intestine.

These tests (summarized in Chart 3) indicate that blood liberated in the stomach would be digested and absorbed to a considerable extent on passing through the intestine so that a considerable hemorrhage might occur before a positive test for blood would be obtained in the feces. On the other hand,

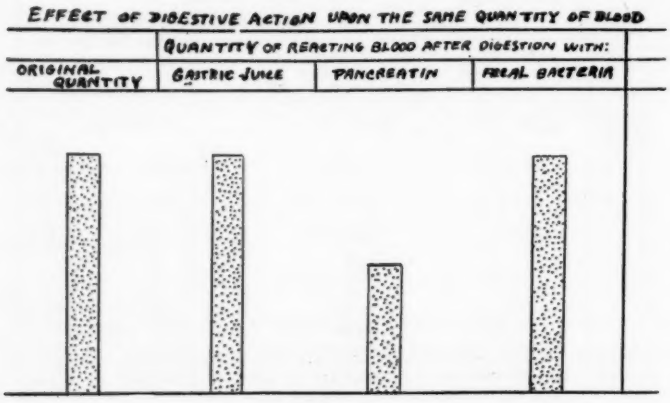
EFFECT OF DIGESTIVE ACTION UPON THE SAME QUANTITY OF BLOOD
QUANTITY OF REACTING BLOOD AFTER DIGESTION WITH:
ORIGINAL QUANTITY
GASTRIC JUICE
PANCREATIN
FECAL BACTERIA

Chart 3.

blood liberated in the lower part of the small intestine or in the colon is not appreciably acted upon, and therefore a much smaller amount would suffice to be detected by a stool test.

To test these assumptions we attempted to liberate the blood in the lower intestinal tract. One would expect to find that a much smaller amount would give a positive reaction in the feces. Dried blood was used, administered in stearic acid coated capsules. Two tenths gm. of KI administered in these capsules appeared in the saliva only after three to four hours. This would allow the blood to be partially acted upon in the intestine, but to a lesser extent than was permitted by the previous method.

The results of these tests are shown in Chart 2. Even though the stearic acid capsulation only partially reduced the intestinal digestion of the blood, there was a reduction of the quantity of blood required to give a positive stool reaction, to 1 gm. (1.2 per cent weight of feces). It is to be assumed that a liberation of quite a small amount of blood in the lower small intestine or colon would result in a positive test in the feces. No suitable method was arrived at for the liberation of the blood so far down in the alimentary tract, so that the latter assumption could not be proved.

VI. *Effect of Diet.*—Many foods give a positive reaction to the benzidine test before ingestion. This is true of meats especially, and it is interesting to know to what extent the ingestion of these articles will affect the test for blood in the stools. The test-negative diet employed in the previous experiments was also used here. To the daily diet the following were added with the results given below:

25 gm. dried beef	positive
250 c.c. meat base vegetable soup	negative
100 gm. chicken	"
1 lamb chop	"
70 gm. hamburger	positive
70 gm. liver (fried)	"
75 gm. roast beef	"

It should be noted that these rather incomplete diet tests were performed on a single subject, and, though he was apparently normal, there are individual differences in digestive power. The above diets would probably not be positive in most cases, but they serve to emphasize that the diet of a patient should be inspected before a positive test for occult blood can be unquestioned. There seems to be no general agreement upon the relation of diet to the test and further experiments are necessary. One worker after an experiment conducted "for eleven consecutive days" found no positive reaction, even after the ingestion of two ounces of boiled ox blood.

To be sure that a positive benzidine test in the stool is due to blood it seems advisable to at least require that all meat be omitted from the patient's diet for one or two days preceding the test. The following have been found to give stools that are consistently negative: milk, cheese, fresh eggs, well-cooked potatoes, butter, dry breakfast foods, baked custard, and most fruits. The patient when on the diet ingests a capsule containing 100 mg. of carmine, which gives a distinctive color to the stool. A portion of the colored stool is then tested for occult blood. A positive reaction under these conditions is of definite diagnostic importance.

SUMMARY

1. Under the conditions of these experiments the ingestion of 3.5 gm. of blood results in a positive stool reaction to the benzidine test. One gram of blood in a stearic acid capsule also gives a positive reaction.

2. In vitro, pancreatic digestion reduces the reacting power of blood to one-half the original.

3. Four hundredths per cent of fresh blood causes the stool to react positively. The liberation of this percentage of blood in the lower intestine should theoretically result in a positive test for occult blood, but this was not proved.

4. To obtain reliable results the patient should be placed on a meat-free diet for two days preceding the test.

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BENCE-JONES PROTEIN IN BLOOD SERUM LEADING TO DETECTION OF MULTIPLE MYELOMATOSIS*

Report of a Case

By J. J. Short, M.D., and J. R. Crawford, M.D., New York

ON REMOVAL of sera from the inactivating water-bath for Wassermann test, one serum was noticed to contain a solid, gelatinous, white precipitate. This serum was notably clear before inactivation. Because of the temperature at which the precipitate had formed (56° C. for thirty minutes) the presence of Bence-Jones protein was suspected. Further examinations of the patient's urine revealed the presence of large quantities of Bence-Jones protein; these were followed by thorough x-ray examinations of his skeletal system which revealed in the cranium the usual pathologic changes found in myeloma. Details of the case follow:

History.—W. W., male, aged fifty-two, born in the United States, real estate broker, married, applied for medical examination on March 26, 1927. His chief complaints were "sluggishness of intestines" and "weariness." Family history was irrelevant. Past history was that he had had chorea and typhoid fever, both with complete recovery. Habits were good except for the use of 6 cigars and 6 pipefuls of tobacco daily, little exercise, too free use of salt and pepper, with little fruit or vegetables in diet.

Physical Examination.—The important findings were as follows: A large-framed man, height 70 inches, weight 180¾ pounds, appeared decidedly ill, worried, tired. Skin showed moderate pallor; eyes, ears, nose, throat and teeth were essentially negative; pulse 72, regular; blood vessels slightly thickened; blood pressure 98/60; heart normal in size, sounds lacking in tone; lungs negative; abdomen negative; reflexes negative; mental state apprehensive; extremities negative.

Laboratory Findings

Hemoglobin.—Eight and eight-tenths gm. per 100 c.c. (equals 52 per cent, Williamson's standard[1]); erythrocytes, 2,828,000; leucocytes, 6,000; color index, 0.91; polynuclear neutrophiles, 57 per cent; lymphocytes, 35 per cent; endotheliocytes, 6 per cent; eosinophiles, 1 per cent; basophiles, 1 per cent. No parasites found.

Urea Nitrogen, 15.8; nonprotein nitrogen, 35.4; uric acid, 5.2; creatinine, 1.8; sodium chloride, 5.98; sugar, 105; cholesterol 150 mg. per 100 c.c.

Wassermann test was negative with alcoholic and cholesterolized antigens.

Routine Urinalysis.—Single specimen, amber, turbid, specific gravity 1.030, reaction acid, albumin trace, sugar negative, few epithelial cells, a little mucus and amorphous material. (In this examination the Robert's test was used for albumin and the Bence-Jones protein was not detected.)

Gastric Analysis.—Free HCl, 40; combined HCl, 16; organic acids and acid salts, 8; total acidity, 64; lactic acid, 0; occult blood, 0; microscopic, negative.

Feces.—Essentially negative, except on one occasion a definite reaction for occult blood was obtained by the benzidine test which was not explained at the time. On a subsequent specimen the test was negative.

Roentgen Examination

After obtaining the characteristic reaction for Bence-Jones protein as noted above, numerous roentgenograms of the bones were made and reported as follows:

*From the Laboratories and X-Ray Department of the Life Extension Institute, New York.
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Examination of the entire skeletal system showed no changes suggesting multiple myeloma with the following exceptions: In the films of the cranium were found, involving some of the bones of the cranial vault, rounded areas of diminished density suggesting the possibility of multiple myelomatosis. In the bones of the right leg there was evidence of slight periostitis involving the tibia, and to lesser extent the tibia of the left leg.

Unfortunately, several of the better original films were given into the possession of the patient and never recovered. One view of the cranial vault is reproduced in Fig. 1.

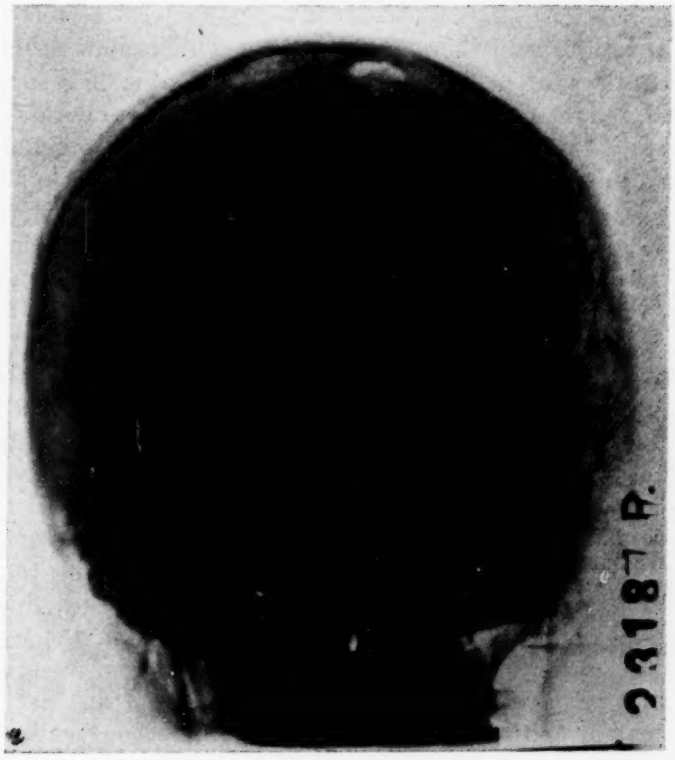

Fig. 1.—Posteroanterior view of cranium showing isolated areas of diministed density at the apex of the cranial vault.

COMMENT

The patient continued at his work as real estate broker until about one month before his death in the spring of 1928 when he was confined to his bed. The immediate cause of death was extreme asthenia resulting from his progressive anemia. There were slight improvements in the blood count following several blood transfusions but the remissions were only temporary.

The two outstanding features of this case are the rather unique way in which the clue to the condition was first discovered and the degree of anemia incident to the slight degree of bone involvement. It would appear that the anemia was due to some inhibitory influence upon the marrow of the other bones, resulting from the disease process, as no extensive structural bone marrow change could be demonstrated.

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THE USE OF SODIUM CITRATE AS ANTICOAGULANT IN THE CHEMICAL EXAMINATION OF BLOOD*

By S. L. Leiboff, M.A., and I. S. Witchell, M.D., New York

IN THE chemical examination of blood, potassium oxalate is most widely used as an anticoagulant. It is the general impression that sodium citrate cannot be used, for the reason that it supposedly interferes with deproteinization, especially when tungstic acid is used as the protein precipitant. It is further claimed that the citrate interferes with the determination of uric acid.[1]

A study of the effect of potassium oxalate and of sodium citrate upon some of the chemical constituents of the blood was undertaken for the following reason: it is our intention to collect data on the distribution of calcium between corpuscles and plasma, and also the relation of calcium to other constituents of the blood. There is a question as to the presence of calcium in corpuscles; thus, Abderhalden[2] who examined the blood of the cow, bull, sheep, horse, pig, rabbit, dog, and the cat, found no calcium in the corpuscles. He did not, however, examine the corpuscles of human blood for calcium. Marriott and Howland[3] also claim that there is no calcium in the corpuscles of human blood. They base their conclusion on their finding that whole blood contains approximately only half as much calcium as does serum. In opposition to this view are the following findings: Schmidt[4] claims to have found about 33 per cent of the calcium present in the corpuscles of human blood. Cowie and Calhoun[5] reported the presence of calcium in the corpuscles of human blood in concentrations only somewhat smaller than in serum. Jones,[6] and Jones and Nye[7] give figures ranging from 5.0 to 8.7 mg. per 100 c.c. for the calcium content of the corpuscles of infants and young children.

It is obvious that oxalated blood cannot be used for the determination of calcium. Sodium citrate forms soluble calcium citrate and is the ideal anticoagulant with respect to calcium determination.

For the sake of economy it is desirable that the calcium determination be done on the same samples of blood that are being sent to the laboratory for routine chemical examination. Our routine examination consists in the determination of nonprotein-nitrogen, urea-nitrogen, uric acid, and sugar. Other constituents are determined when requested.

EXPERIMENTAL

Two hundred c.c. of blood were collected from a patient with pulmonary edema into two containers, 100 c.c. into each. One of these contained 30 drops of a 20 per cent solution of potassium oxalate, and the other contained 30 drops of a 25 per cent solution of sodium citrate. The concentration of the two salts was adjusted according to their molecular weights.

Complete prevention of coagulation was effected with both salts.

*From the Biochemical Laboratory of Lebanon Hospital, New York.
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The bloods were immediately taken to the laboratory and six 10 c.c. portions of each blood were pipetted into flasks. Varying amounts of oxalate and citrate were added to the respective bloods. To each sample was then added 70 c.c. of water and 10 c.c. of each, 10 per cent sodium tungstate, and two-thirds normal H_2SO_4. The following determinations were done on the filtrates: nonprotein-nitrogen, by the method of Koch-McMeekin;[8] urea-nitrogen by the method of Folin and Denis;[9] uric acid by the method of Folin;[10] creatinine by the method of Folin;[11] sugar by the method of Folin and Wu.[12] The results are shown in Table I.

TABLE I

NO. BLOOD	ANTICOAG. ADDED	N-P-N		UREA N.		URIC ACID		CREATININE		SUGAR	
		OXL.	CTR.	OXL.	CTR.	OXL.	CTR.	OXL.	CTR.	OXL.	CTR.
1	None	36.5	36.0	16.2	15.9	3.8	3.9	1.7	1.6	113	111
2	2 dr.	35.9	36.2	16.6	16.1	3.6	3.6	1.7	1.7	110	109
3	5 "	36.6	36.7	16.1	15.8	3.7	4.1	1.6	1.5	112	109
4	10 "	36.4	37.2	16.9	16.4	*	3.8	1.8	1.6	109	106
5	15 "	36.0	41.0	17.2	17.0	*	3.7	1.5	1.8	105	102
6	1 c.c.	37.1	42.4	16.9	17.0	*	4.0	1.6	1.7	104	100

*The solutions became very cloudy and could not be read in the colorimeter.

DISCUSSION

We encountered no difficulty in obtaining clear filtrates in the presence of citrate with the reagents used. In this respect the citrate and the oxalate behaved alike.

The citrate not only does not interfere with the determination of uric acid by the method used, but as is shown by the table, it is superior to the oxalate. The last three tubes containing large amounts of oxalate became very cloudy on adding the reagents and very little color developed, while all the tubes containing citrate, even those containing the largest amount used in the test, remained very clear and developed a good color.

As for the other constituents determined, in the presence of excess citrate the nonprotein-nitrogen fraction is definitely increased, while the amount of sugar is somewhat decreased. Practically, this is of no significance, since the amount of citrate producing such faulty results is far in excess of the amount that may ever be used for collecting blood.

The effect of sodium citrate on the chemical constituents of the blood was further tested on a number of bloods taken for routine examination. These

TABLE II

NO. BLOOD	N-P-N		UREA N.		URIC ACID		CREATININE		SUGAR	
	OXL.	CTR.	OXL.	CTR.	OXL.	CTR.	OXL.	CTR.	OXL.	CTR.
1	28.3	28.8	11.7	11.6	1.5	1.6	1.2	1.3	96	95
2	31.7	31.6	15.1	15.0	2.2	2.4	1.6	1.6	109	107
3	30.0	29.7	14.4	14.1	2.7	2.5	1.4	1.4	116	114
4	53.5	54.2	36.3	35.8	7.2	7.5	2.9	3.0	117	119
5	30.4	29.9	14.0	14.3	1.9	1.8			83	80
6	35.2	35.7	16.2	16.6	2.7	2.9	1.4	1.4	227	221
7	26.2	25.9	11.3	11.1	1.1	1.2	1.0	1.0	79	81
8	78.0	79.3	43.5	42.8	8.7	9.0	3.6	3.7	124	121
9	33.4	33.0	15.0	14.4	3.2	3.1			188	185
10	37.4	38.0	17.5	17.1	3.6	3.7	1.8	1.7	92	93

bloods were collected in duplicates; one portion in potassium oxalate, and the other portion in sodium citrate. The bottles for collecting the bloods were prepared by introducing three drops of 20 per cent oxalate solution into each bottle, and dried at a temperature of about 100° C. Another set of bottles was prepared containing three drops of 25 per cent solution of sodium citrate. These bottles were dried in the incubator at 37° C. overnight. High temperatures should not be used for drying the citrate.

The above-mentioned five determinations were then performed and the results compared. Table II shows that the results are quite satisfactory.

SUMMARY

1. A comparative study was made of the effect of potassium oxalate and sodium citrate upon the determination of some of the chemical constituents of the blood.

2. Sodium citrate may be used as the anticoagulant in blood for these determinations. With the amounts of citrate used no difficulties are encountered.

3. The complete precipitation of blood proteins is produced.

4. In the determination of uric acid by the method of Folin better results are obtained with the citrate than with the oxalate.

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THE WASSERMANN REACTION OF HUMAN MILK

A Comparison of Blood and Milk Tests in 107 Cases

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THE performance of the Wassermann test on the milk suggests itself as a convenient method of detecting the presence of syphilis in puerperal women. To study the reliability of this test, specimens obtained from 125 mothers from the third to the seventh day postpartum were subjected to the Wassermann test in an institution in which the blood is examined routinely. In 107 instances a comparison of the Wassermann reaction in the blood serum and in breast milk showed close parallelism. The remaining 18 are not considered here since the reports of their blood are not available; of their milks, 1 was three-plus and 17 were negative.

REPORTS OF EARLIER INVESTIGATORS

Bab[1] in 1907 performed Wassermann tests on the breast milk of three syphilitic and one nonsyphilitic woman and observed that the reaction was positive in the syphilitic patients and negative in the other woman. Later Guicciardi,[2] Bauer,[3] and Bar and Daunay[4] reported a few cases in which a positive reaction was obtained from the breast milk of women known to be syphilitic.

Bar and Daunay performed the Wassermann test on the breast milk of 8 women with florid syphilis. In 5 cases the reaction was positive; in two, doubtful; and in one, negative. In 15 nonsyphilitic women the reaction was negative.

Thomsen[5] in 1909 reported that in 9 syphilitic women who began to nurse their infants, the reaction of the breast milk was definitely positive during the first two days after labor. Then it began to diminish in intensity and usually became negative after the fifth or sixth day of the puerperium. In 8 syphilitic women who did not nurse their infants the positive reaction persisted until it was no longer possible to obtain milk for examination (8 to 14 days). In some of these women, known to be syphilitic, the Wassermann reaction of the serum was negative. In 82 nonsyphilitic women who were nursing their infants, the reaction was negative when the milk was tested from five to thirty-five days after labor. However, in 9 of 46 nonsyphilitic women whose milk was tested in the first two or three days of the puerperium, the reaction was slightly positive until the fifth or sixth day. In none of these cases was the reaction of the serum positive. In 8 nonsyphilitic women who did not nurse their children, the reaction of the breast milk was uniformly negative.

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In a group of 28 puerperal or pregnant women, Rojas[6] in 1919 observed a positive Wassermann reaction in the milk or colostrum and also in the blood in 7 cases. Seventeen of the patients had been delivered within five days and 11 were in the last two months of pregnancy. In three of the positive cases, the reaction was more pronounced in the milk than in the blood. In 6 cases, there was a positive reaction in the milk and a negative one in the serum. The false positive reactions obtained in the colostrum may, in my opinion, be attributed to the richness of that substance in globulin or to the anticomplementary substances, if the milk had not been heated.

Examining the breast milk of 40 women, 16 of whom were known to hav syphilis and 15 to be free from the disease, Rusca[7] in 1919 observed a con stantly negative reaction in the mammary secretion from normal women from the second to the ninth month of pregnancy. In the secretions obtained from the syphilitic women there was a strongly positive reaction, which tended to disappear under treatment and parallel the reaction in the blood. Rusca's further study,[8] reported in 1921, confirmed the presence of syphilitic reagin in breast milk even in advanced lactation. He expressed the view that the milk reaction is not so sensitive as that of the blood, but that a positive reaction in breast milk indicates grave and active syphilis.

Klauder and Kolmer[9] in 1921 performed Wassermann tests on the breast milk of 19 syphilitic women, all of whom had a positive reaction of the blood. In the majority, the reaction was four-plus with three antigens. The majority of the patients were untreated and presented no clinical evidence of syphilis, although some had secondary and late secondary skin lesions.

The milk was obtained at variable periods after childbirth or during the last month of pregnancy. With the technic employed, fresh, unheated human milk was anticomplementary in amounts varying from 0.5 to 0.9 c.c. Tests conducted with one-half or even slightly more of these anticomplementary amounts yielded negative reactions in the milk of all women except three, two of whom were tested within five days of delivery. In one of these two cases the milk was likewise negative before and three weeks after delivery. It is difficult to interpret the findings of Klauder and Kolmer because of the fact that raw milk was used and anticomplementary reactions were obtained, also because in 16 of their 19 cases the milk was examined a month or later after delivery.

In human milk from 89 syphilitic and 21 nonsyphilitic mothers, Cotellessa[10] in 1923 obtained parallel Wassermann reaction with those of the serum. To avoid inactivation the fat was removed by centrifugation. The test was performed with 0.5 and 1 c.c. of milk.

Schwarz and Schubert[11] in 1925 reported parallel Wassermann reactions in the blood and milk of 45 women in the early days of the puerperium. In 17 cases the reaction in the blood was negative and that in the milk was also negative, except for an anticomplementary reaction in two cases. In the remaining patients, who were definitely syphilitic, the Wassermann reaction in the milk corresponded with that in the blood. However, there were some primary infections in which the reaction was positive in the blood but still nega-

tive in the milk. In three cases in which the blood reaction had been reduced by intensive treatment, the milk still gave a strongly positive reaction.

Martin[12] in 1925 noted that he had found the Wassermann reaction positive in the milk in all women with positive blood reactions; but, in women nursing their infants, he observed that the reaction becomes negative in a few days, thus confirming Thomsen's earlier report.

Hackemann[13] in 1926 reported a parallel study of the Wassermann reaction in the milk and serum in a number of cases. In 24 nursing mothers whose blood reaction was positive, the reaction in the milk was also positive until the third to the eleventh day of the puerperium, an average of six to seven days. In 5 nursing mothers whose blood reaction was negative, there was a positive reaction in the milk, which lasted until the third to the fifth day of the puerperium (average 4.5 days). In four cases the Wassermann was negative in both the blood and the milk. In three cases in women who did not nurse their infants, the reaction was positive in the blood and also in the milk until the thirteenth to the twenty-fifth day postpartum. Hackemann believes that the false positives in the milk during the early days of the puerperium are due to the presence of serum globulin.

Lipinski and Keller[14] in 1926 reported a study of the Wassermann test on mother's milk in 200 women, from which they concluded that the Wassermann reaction is positive in the milk of syphilitic women and remains so, as late as the sixteenth day of the puerperium, even in women who nurse their infants. In cases of latent syphilis, they believe, it may be positive even when the blood reaction is negative. In their experience, the blood was never positive when the milk gave a negative reaction. In some cases they observed a false positive reaction during the first two or three days of the puerperium in nonsyphilitic women with negative blood tests; but these false positives could be avoided by employing not more than 0.05 c.c. of milk for the test. In the case of syphilitic women, the reaction was positive with 0.025 and even 0.0125 c.c.

Franken and Rothmann[15] in 1927 reported a study of 225 cases in which the Wassermann reaction of the milk was compared with that of the retroplacental blood and, in some cases, with that of blood obtained in the ordinary way. The Wassermann reaction in the milk agreed with that in the blood in 95.5 per cent of the entire series. In 11 cases in which there were no definite clinical signs of syphilis but the reaction of the arm blood and retroplacental blood was positive, the reaction in the milk was also positive. In 4 cases in which the retroplacental blood was negative but there was either a definite history of syphilis or a positive reaction in the blood obtained from the arm, the reaction in the milk was positive. In three patients under treatment for syphilis, the milk gave a negative reaction. One of them had a positive blood Wassermann. In 8 cases in which there was no clinical evidence of syphilis and the blood test was negative, a nonspecific positive reaction was obtained in the milk. In 5 other cases without clinical evidences of syphilis, there was a nonspecific or anticomplementary reaction in both the milk and the serum. Franken and Rothmann conclude that the Wassermann test on the milk is of no more diagnostic value than that on the blood but that

it is of value as a check on the diagnosis in cases in which the retroplacental blood is not obtained or in which the blood from the arm is not tested at the time of discharge from the hospital. Especially is this true in the detection of latent syphilis. In their experience, the Wassermann reaction in the milk of syphilitic women is most strongly positive from the eighth to the tenth day postpartum.

CLINICAL MATERIAL STUDIED

The milk was obtained from 125 recent colored mothers in a city institution in which the blood Wassermann is performed as a matter of routine. This class of patients was chosen because, by reason of social, economic and educational disadvantages, they would be less likely to resort to early medical aid when infected. Also, even if treatment were started early, this class of patients would be less likely to persist in the treatment sufficiently long to rid themselves of the disease than those in more fortunate circumstances.

Through the efforts of Dr. E. G. Langrock and the courtesy of Dr. F. A. Kassebohm, I was able to obtain milk from the third to the seventh day postpartum. Twice a week the milk was pumped or expressed from the breast. Before obtaining the specimens, the breasts were washed. A clean, sterile pump was used in each case. The specimens were placed in clean, sterilized saltcellar bottles and kept on the ice until examined. Each bottle was labeled with the following data: name, address, age, hospital number, number of children, number of abortions, day postpartum, result of blood Wassermann, result of milk Wassermann, and date when milk was obtained. Due in part to the fact that most of the patients were young primiparae ranging from sixteen to twenty-three years of age, the majority denied abortions. The youngest patient was thirteen years old; the oldest, forty-one.

REMARKS ON TECHNIC

The Wassermann tests on the milk were performed by Dr. A. A. Eisenberg, Director of the Syndenham Hospital Laboratories, who had no knowledge of the blood Wassermann results when he submitted his reports.

Milk may be prepared for the test by shaking with ether and prolonged centrifugalization or by passing it through a Berkefeld filter. The latter method, however, weakens the positive reactions somewhat, probably by holding back part of the reagin.

The technic of Franken and Rothmann,[15] which is almost identical with that of Lipinski and Keller,[14] is to be preferred. The milk is centrifugalized for thirty minutes at high speed, as a result of which three distinct layers were formed. The lowest layer is a thin stratum of grayish sediment; the middle layer, which is the largest, is watery and almost colorless; the upper layer is a narrow stratum of fatty material. A capillary pipette to which a rubber nipple is attached is introduced into the middle layer and the watery fluid drawn off. This fluid is inactivated by heating for thirty minutes in a water-bath and then diluted with normal saline solution in a ratio of 1:5 or 1:10. The amount of diluted fluid used for the test is from 0.05 to 0.1 c.c.

Thomsen's[5] observation that some nonsyphilitic mothers had Wassermann-positive milk is probably explained by the fact that he used noninactivated

milk, as was also the case in 8 of 185 cases reported by Lipinski and Keller.[14] Unheated milk may give a false positive due to the anticomplementary action of raw milk.

It is generally agreed that the milk should be obtained from nursing mothers from the sixth to the ninth day postpartum and that the reaction tends to become negative after the sixteenth day. The explanation probably lies in the fact that in the earlier days of the puerperium the breast secretion is more of the nature of colostrum than true milk, thus accounting for the close relation between its proteins and those of the blood serum. In true milk, on the other hand, the protein constituents are the product of a specific secretion of the mammary glands. Apparently the reagin responsible for the positive Wassermann reaction passes from the blood into the colostrum. This hypothesis would be consistent with the general observation that incomplete or slow emptying of the breast prolongs the duration of the positive Wassermann reaction in the milk. Schwarz and Schubert[11] sometimes observed positive Wassermann reactions in the milk of women who did not nurse their infants as late as the thirty-second day after delivery because, as they believed, local congestion had caused a retention of colostrum.

RESULTS OF INVESTIGATION

The results of the parallel Wassermann tests on blood and milk, respectively, in 107 cases are given in Table I.

TABLE I

COMPARISON OF PARALLEL WASSERMANN TESTS ON BLOOD AND MILK

	NO OF CASES
Both blood and milk negative	86
Both blood and milk 4-plus	3
Both blood and milk 3-plus	3
Both blood and milk 1-plus	1
Blood 4-plus, milk 3-plus	1
Blood 4-plus, milk 2-plus	1
Blood 4-plus, milk plus-minus	1
Blood doubtful, milk 1-plus	1
Blood 4-plus, milk negative	1
Blood 1-plus, milk negative	1
Blood negative, milk 3-plus	1
Blood negative, milk 2-plus	2
Blood doubtful, milk negative	3
Blood negative, milk anticomplementary	2
Total	107

If we drop from consideration the two cases in which the milk was anticomplementary, there was exact qualitative and quantitative agreement in 89 per cent of the cases and substantial agreement in 95 per cent.

In five cases contrary results were obtained. In three instances the milk was positive and the blood negative; in two, the blood positive and the milk negative. The two anticomplementary reactions were due to the fact that unheated milk was used in these cases.

PRACTICAL APPLICATIONS

If the Wassermann reaction of the breast milk of puerperal women closely parallels that of the blood, as the evidence strongly suggests, routine examination of the milk from the sixth to the ninth day postpartum should offer a convenient alternative to the blood Wassermann test. It is simpler and avoids unpleasantness in the case of sensitive patients.

A Wassermann test on the milk of prospective wet nurses, recently delivered, should be performed as a routine. The test is especially useful for women who refuse to submit to repeated blood tests though they may have become infected with syphilis and develop a positive reaction during their wet nursing period. All milk received at the stations should be subjected to the Wassermann test.

In private practice it is a simple matter for the physician to obtain a specimen of milk in suspicious cases and perform the test without the knowledge of the other members of the family. Many patients object to the blood test because it draws searching questions. The significant puncture mark of the Wassermann test has frequently attracted the attention of husbands or others and has thereby precipitated domestic crises. When the reaction in the milk is positive, a blood Wassermann should always be obtained for confirmation.

SUMMARY

Parallel blood and milk Wassermann tests were performed upon 107 colored puerperal patients in a city institution. There was substantial agreement in 95 per cent of the cases, and exact qualitative and quantitative agreement in 89 per cent. In three instances the milk was positive and the blood negative; in two, the blood positive and the milk negative.

The Wassermann reaction remains positive in the milk of syphilitic women until about the sixteenth day, when it begins to weaken and gradually disappears. False positive reactions can largely be avoided by inactivating the milk and using smaller quantities for the test. Apparently the reaction is due to the presence of the reagin in the colostrum, whose composition more nearly approximates that of the blood serum than does the true milk.

Routine examination of the milk from the sixth to the ninth day postpartum offers a convenient means of employing the test in maternity practice.

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303 West 106th Street.

A METHOD FOR SERIAL SECTIONS IN CELLOIDIN*

By E. P. Fowler, Jr., New York City

IN THE course of a series of experiments in this laboratory requiring histologic sections of petrous bones, it was found that the paraffin methods for serial sections and the usual celloidin methods of staining each section individually were unsatisfactory. To insure a uniform staining reaction and to cut down the time necessary to make a long series of sections the following method was devised.

The apparatus may be quickly improvised in any laboratory. Several ordinary glass slides are prepared by fastening a single loop of 22 gauge brass wire about 2 cm. from one end of each. The wire must be flush with the glass on both sides. This is accomplished by bending the wire at right angles in the proper places, so that it will fit the slide, and by twisting the free ends of the wire as tightly as possible without breaking the slide. It was found that if all the wire loops were made on one slide and then slipped on to other somewhat larger slides (slides usually are slightly irregular in width) the loops could be made to fit more snugly and fewer slides were broken. One of these wired slides should be prepared for every section that is to be cut from a single block.

A rack is then made out of 14 gauge copper wire which will hold a number of these slides when they are stacked on top of one another. A rack which holds about 35 to 40 slides is shown in the figure. This size was most convenient to handle but if more sections are desired, a deeper rack can be made, and very deep jars used for staining.

The general idea for making the rack can best be ascertained from the figure. The joints are held together with fine wire and need not even be soldered if they are well bound. The tissue to be cut is embedded in the usual way, except that the celloidin block is trimmed and mounted rather

*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.
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longer on one end than is ordinarily necessary. This will leave a tab of celloidin on each section cut.

The sections are cut with the knife at an angle, and with edge and block swimming in 80 per cent alcohol. As each section is cut, it is flattened out on the knife blade, but always thoroughly covered by the excess alcohol there. When flat, it is removed with forceps to one of the prepared slides. The tab end of the section is placed over the wire (see figure) and pressed down on the slide with the ball of the thumb, so that it will stick there temporarily. This slide with the section adhering is then inverted and put in the rack which has one empty slide already in place. The slide is placed so that its wire is at the opposite end of the rack to the first slide. The rack with the two slides is lowered into a beaker of 80 per cent alcohol immediately after

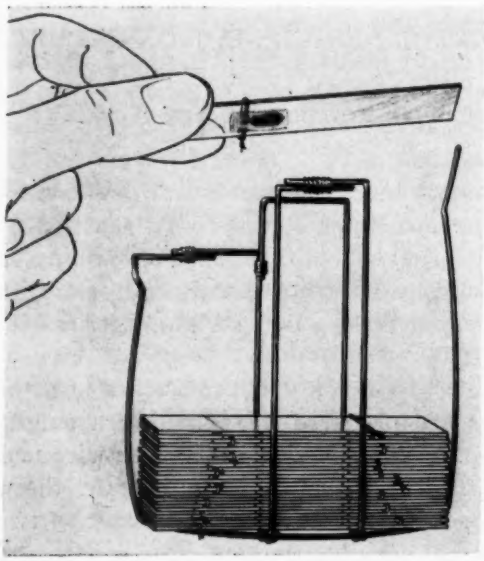

Fig. 1.—Showing method of building rack, placing sections on wired slides and placing slides in the rack.

the above operation. The second section is then cut and placed on a wired slide. The rack is raised and the slide put in place with the section down and the wire on the opposite side of the rack from the preceding slide. The whole is then again lowered into the alcohol. This process is repeated until the rack is full, always inverting the slides and always staggering the wires so that the pile is flat, and each section is held in place between a wire above and a slide beneath. When the rack is full, or the required number of sections has been cut, a couple of extra wired slides are placed on top, so as to provide enough weight to keep the upper sections in place. If desired a special weight may be used for this purpose.

Before staining, the rack is raised and lowered a few times in the alcohol, so as to be sure that all the sections are fast. They should be secure if all the wires have been properly put on. If they are not secure, corners of some of the sections will be seen slipping out from between the slides. The serial

numbers of such sections can be ascertained by counting the spaces either from the bottom or the top of the pile. These numbers should be noted, and the sections gently pulled out, placed on other wired slides and put on top of the pile, under the weights.

The whole rack is now run through the desired stains and solutions in tall wide-mouthed jars. From the last jar the sections are removed in order from their wired slides and mounted in balsam in the usual way. If the excess celloidin does not conveniently fit under the cover glasses it can easily be cut off.

The mounted sections are now numbered and the sections which slipped out in the alcohol (if any) carefully put into their proper places. The result is a series of sections all equally stained.

AN ATTEMPT AT MICROSCOPIC OBSERVATION OF LIVING TISSUE*

By William C. Chaney, M.D., and Leonard G. Rowntree, M.D.
Rochester, Minn.

THIS study of the histology of living tissue was suggested by the work of Krogh on the capillaries and that of Richards on the glomerulus of the kidney. The idea underlying this work was the application of their methods to the study of other living tissue, particularly the liver and the spleen.

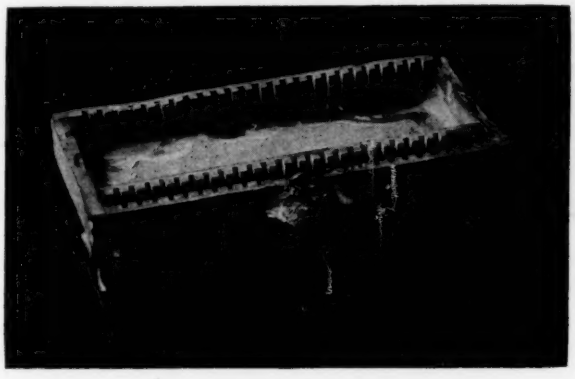

Fig. 1.—Substage condenser.

Investigations began with the spleen. It was realized that a relationship exists between many tissues and that the sharing of function by various organs is incident to the presence in them of a common type of cell. The study of this common type of cell or tissue, the so-called reticulo-endothelial system, claimed primary interest in both the liver and the spleen.

As technical difficulties were overcome and photographs of living cells

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were obtained, it became evident that a sufficient amount of information could be secured through these methods to make them of practical importance.

From the beginning it was evident that there were certain limits to the work which were concerned largely with blood supply, distribution of tissues, thickness of tissues, light and heat. A method had to be used which would permit of detailed microscopic study of individual cells such as could be seen with a magnification of almost 1000 diameters and at the same time permit the maintenance of physiologic conditions. This was accomplished by using a substage condenser projecting above the stage of the microscope, preferably fixed within the board on which the animal was held (Fig. 1). The tissue to be studied was then placed directly on the condenser and kept moist by

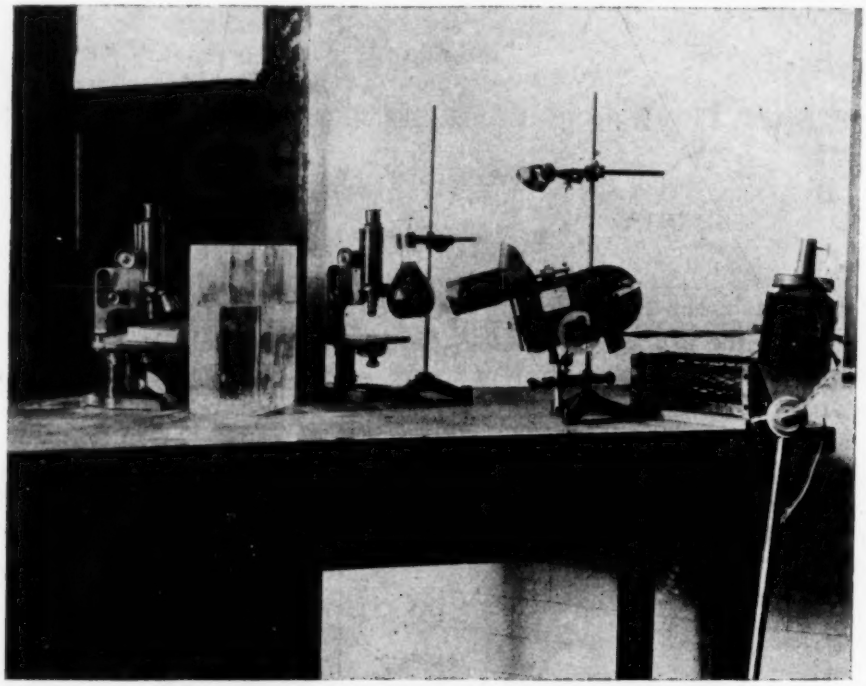

Fig. 2.—The small arc light is directed toward the jar of alum water. The board on the stage of the microscope contains the condenser. The Florence flask is used whenever reflected light is desired.

the salt solution which filled the moat around the condenser. The body of the animal was brought into close proximity to the condenser to prevent tension on the tissue under study. Very young mice and young frogs were used and found to be much better than older ones.

Intense light was essential to success. This was best supplied by a small arc, the rays from which were passed through a glass jar containing a saturated solution of alum to filter out the heat. For purposes of differentiating the cells, colored solutions such as copper sulphate or potassium dichromate, depending on the tissue under observation, were used in the alum solution. Glass filters of various colors were also used (Fig. 2).

The use of transmitted light, by the method just described, gave the best results, but because of the thickness of certain tissues this method is not

always applicable. Reflected light has been used in a good many instances, success depending on placing the rays of light at a point on the tissue such that the angle of incidence is quite acute. An ordinary condenser may be used for this purpose or a 300 c.c. Florence flask filled with alum solution. There are at least two objections to using reflected light: it shows mostly surface detail, and it is difficult to obtain the proper angle of light when the higher magnification is employed, such as a 4 mm. objective.

Thus far our interest has centered around two problems: the function of the reticular cells in the reticulo-endothelial system, first described as "macrophages" by Metschnikoff and later by Evans, and the relationship existing between the sinuses, capillaries, and venules of the spleen.

Effort was made to show the process of phagocytosis by these reticular cells to determine whether they are more active in one organ or another and how they behave toward particulate bodies, cells of other animals, parasites of the blood stream, and injured cells of the same animal. As is well known, when dialyzed india ink is introduced into the circulation of a frog it is taken up by the endothelial cells of other tissues. The particles of the ink seem to stick to the endothelial cells very much as though these cells were droplets of honey. The free end of the ink particle may then wave to and fro in the blood stream, may be subjected to constant bumping by erythrocytes, may be dislodged or may finally come to occupy a place within the cytoplasm of the cell. In the frog the liver appeared to take up more of the ink than any other tissue, the spleen being second, then the lungs, then the capillaries of the intestinal tract and finally the kidneys.

In unstained tissue difficulty is of course encountered in differentiating the types of cells. It is evident that the minute cytologic study of living tissue and cells will necessitate the use of vital stains as a means of differentiation. The application of vital dyes even to the surface of an organ is of marked value in the differentiation of cells. The spleen of the white mouse, for example, when viewed without staining, appears as a homogeneous structure, although here and there a blood stream may be discerned. If, on the other hand, methylene blue is injected into the peritoneal cavity an hour or so prior to the observation, the cells of the spleen will have taken up such an amount of the dye as to make differentiation of cells seem a possibility by this method.

With reflected or transmitted light the blood vessels are usually recognized readily because of the constant movement of the cells. The frog's corpuscles, particularly, are admirably suited for the study of cells in the blood stream because of their size and their large nuclei. Many attempts have been made to examine blood cells after they had been stained with vital dyes and returned to the circulation of the same animal, but without significant success, since the blood plasma, or the liver, or some other agent, has been effective in the discoloration or the destruction of the cells to a large degree.

Arterial capillaries and veins have been identified in both liver and spleen. It is difficult to decide whether or not splenic sinuses have actually been visualized. In the spleen large channels have been seen. Alternation of function of venous capillaries has been observed in the spleen of the frog, corresponding to the phenomenon observed by Krogh in the muscles of frogs.

AN AUTOMATIC FILTERING DEVICE*

By Manuel G. Gichner, M.D., Baltimore, Md.

IN LABORATORY practice it is often necessary to recover the precipitate from a large volume of fluid upon a small filter paper, as in the preparation of Romanowsky stains or in the preparation of protein antigens by extraction

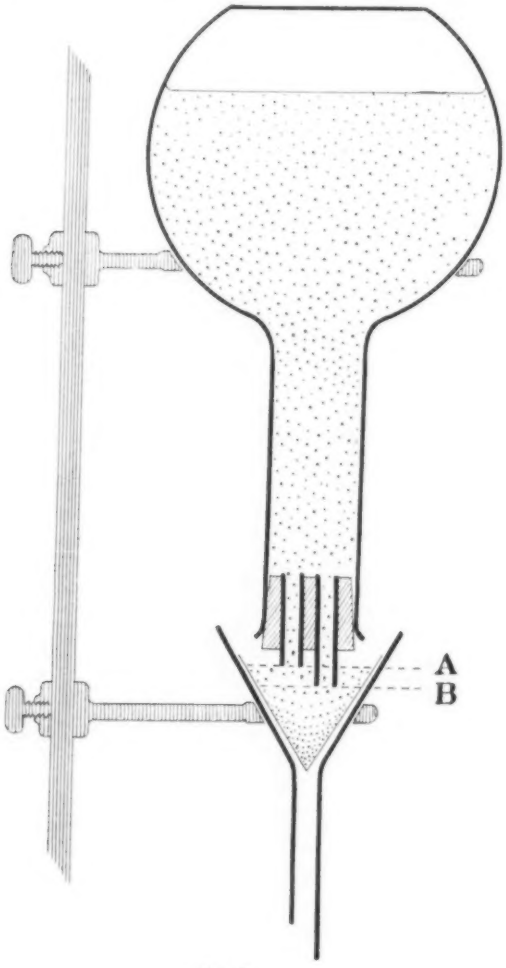

Fig. 1.

with dilute sodium hydroxide and precipitation with dilute hydrochloric acid. The procedure frequently necessitates an expenditure of time and attention out of all proportion to the importance of the end accomplished. This apparatus was devised to facilitate such filtrations.

*Received for publication, December 18, 1928.

The accompanying drawing is self-explanatory. Two lengths of glass tubing are inserted into a two-hole stopper. The difference in length of the tubes should be slightly greater than the diameter of the lumen, and their inner ends should be flush with the inner surface of the stopper. The stopper is inserted into a flask containing the material to be filtered and the flask is inverted over the filter. Properly adjusted ring supports hold flask and filter in the desired position. Fluid escapes, filling the filter to the level of the shorter tube (level *A*), when no more air can enter the flask and the flow ceases. As filtration proceeds, the level of fluid in the filter falls until a level is reached at which air again enters the shorter tube (level *B*), and the fluid rises to the previous level (level *A*), when flow again ceases. It is sometimes necessary to secure the stopper to the flask with stout cord, especially if large volumes (five liters or more) are handled at one filtration.

It seems that so simple a device must have been previously described, but I was unable to find it in a cursory survey of the literature. Several very experienced chemical technologists whom I consulted believe it to be a new apparatus.

2426 Eutaw Place.

A NEW HEAD-HOLDER FOR THE GUINEA PIG BOARD*

By Susan Griffith Ramsdell, M.A., Philadelphia

GUINEA pig boards in general use in most laboratories have been of the simplest improvised sort, since the usual catalogue offerings have been of the complicated, multiple-purpose type, and therefore prohibitively expensive. The exception has been that devised by Dr. A. F. Coca of the Cornell Medical School; from this model, the one herewith presented varies in the substitution of a different type of head-holder, the original spring-bar for the mouth being replaced by a set of three sliding brackets, adjusted with wing nuts to fit tightly around the jaws, thereby holding the head firmly without discomfort. (Fig. 1, *A* and *B*.) It has the further advantage that the animal can be held equally well in the face-down position. There is thus provided a board which answers certain definite laboratory needs in an operating board:

1. For the greatest possible immobilization of the animal.
2. For dispensing with the use of an assistant.
3. For unlimited use in the various technical procedures on this animal.
4. For an inexpensive, simple, various-use apparatus.

It has been found of particular advantage in the following technical procedures:

1. Heart puncture, where the usual high mortality is due largely to sudden movement of the animal while the needle is in or around the heart.

2. Jugular puncture where quietness of the animal is even more important for success in entering the vein.

Received for publication, October 26, 1928.

*From the Research Institute of Cutaneous Medicine, Philadelphia.

3. Ear vein injection of Rous[1] which is of the greatest use in many instances, but which has been possible hitherto only with the aid of an assistant (Fig. 2).

4. Intracutaneous skin-testing; and many other uses which are as readily apparent.

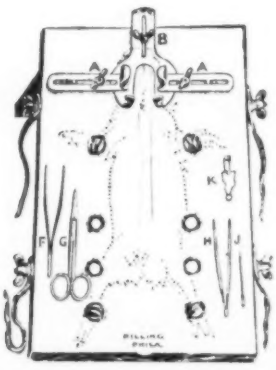

Fig. 1.—The guinea pig board of Coca with a new type of head-holder.

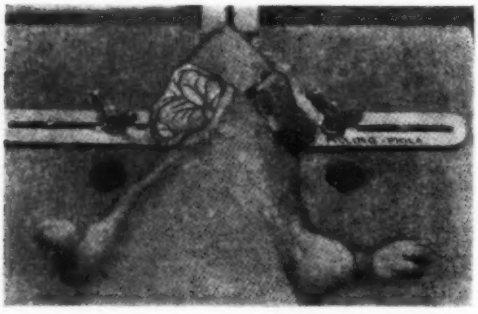

Fig. 2.—Position for ear vein injection. The left ear has been shaved.

In addition, with this device one may carry out the usual laboratory procedures upon white rats without the aid of an assistant.

The George P. Pilling and Sons Company, Philadelphia, has been kind enough to carry out my designs.

[1]Rous, P.: J. Exper. Med. 27: 459, 1918.

A MODIFICATION OF THE BELL TYPE OF STETHOSCOPE*

By Burgess Gordon, M.D., Philadelphia

A STETHOSCOPE with a metal or hard rubber chest piece may cause discomfort to patients who are nervous and emaciated. If pressure is applied to the instrument, which is often necessary when the ribs are prominent, the discomfort is considerably increased.

A modification of the bell type of stethoscope (Fig. 1) consists of a circular metal base 3.5 cm. in diameter to which is attached a projecting soft rubber ring. On the reverse side the central portion is extended upward to serve as a finger rest and point of attachment for the steel and rubber transmission tubes which protrude at right angles from the chest piece.

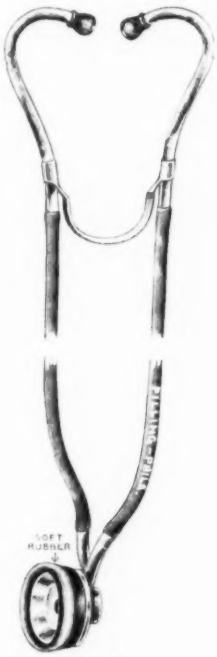

Fig. 1.

The rubber ring is warm, yields to the irregularities of the chest wall, and due to adherent qualities tends to reduce the passage of "hair noises" and other extraneous sounds. The chest piece, owing to shallow construction and the use of side rather than vertical tubes, permits examination without disturbing the position of the patient in bed.

*From the Department for Diseases of the Chest, Jefferson Hospital.
Received for publication, October 26, 1928.
The stethoscope is manufactured by George P. Pilling and Son Company.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CARBON MONOXIDE: A Simple Test for the Recognition of. Schwarzacher, W. Deutsche Ztschr. f. d. ges. gerichtl. med. **12**: 510, 1928.

In three tubes set up:

1. CO-blood diluted 1:200 with water.
2. Normal blood.
3. 0.2 per cent cuprous sulphate.

Tube 1 is superimposed on tube 3 (blue against a light background) with the control blood at the left (yellow against a light background). If observed so that the control blood is superimposed upon the copper solution it appears green and the CO-blood directly to the right of it is red.

GASTRIC ANALYSIS: Minim Method for Analysis of Gastric Content, Einhorn, M. New England M. J. **198**: 941, 1928.

The following method permits the qualitative and quantitative analysis of extremely small amounts.

The same pipette should be used throughout, the tip being bent at an angle of 60 so that, when held horizontally, the same size drop is always delivered.

Solutions:

Yellow Solution 1: 1 minim of Topfer's solution to 5 c.c. of N/100 NaOH.
Yellow Solution 2: 2 minims of Topfer's solution to 5 c.c. of N/200 NaOH.
Yellow Solution 3: 3 minims of Topfer's solution to 8 c.c. of N/400 NaOH.
Scarlet Solution 1: 1 minim of phenolpthalein to 12 c.c. of N/100 NaOH.
Scarlet Solution 2: 1 minim of phenolpthalein to 7 c.c. of N/200 NaOH.
Scarlet Solution 3: 3 minims of phenolpthalein to 9 c.c. of N/400 NaOH.

For every drop of No. 1 solution (yellow and scarlet) the final reading is increased by 10; for every drop of (yellow and scarlet) No. 2 solution, the final reading is increased by 5; and for every drop of either No. 3 solution, the final reading is increased by 2.5.

Method:

Free HCl.

Place one clear drop of gastric contents on a porcelain plate or in a paper tray. Add drop by drop yellow Solution 1 until a reddish-yellow color appears. Then change to yellow Solution 2 and 3 and add these successively until the specimen is exactly the same color as the yellow solution.

(If this occurs at once the specimen contains no free HCl. In such case, the examination of a second drop should be made using yellow Solutions 2 and 3 to measure any small amounts which may be present.)

For each drop of Solution 1 add 10; add 5 for each drop of Solution 2, and 2.5 for each drop of Solution 3.

Thus using 2 drops of No. 1, 1 drop of No. 2, and 1 of No. 3, the reading is: Free acid = 27.5.

Total Acidity: The same technic is followed using the scarlet solutions until the specimen is almost the same color as the solution. Using the same computation above gives directly the amount of total acid.

RENAL FUNCTION: A New Test, Brain, R. T., and Kay, H. D. Quart. J. Med. 1: 203, 1929.

The test described is based upon the excretion of extra phosphorus in the hour following the intravenous injection of 550 mg. of sodium glycerophosphate. In normal individuals at least 150 mg. is excreted. The technic is as follows:

At about 6 A.M. the patient is given a light breakfast, devoid of fat, in order to prevent, as far as possible, a lipemia. At 8:30 A.M. he is told to empty the bladder, and at 9:30 is given 400 c.c. of water. At 10:30 A.M. the first (two hour) specimen of urine is collected and measured, and a portion is taken for total phosphorus analysis. (At this stage a resting blood sample may be taken, if required.) Ten c.c. of glycerophosphate in approximately 50 per cent sterile solution is then slowly injected into the median basilic vein from a record syringe. The time is now taken. Exactly one hour after the end of the injection, the second sample of urine is collected (precautions being taken to empty the bladder completely) and measured. After the proteins have been precipitated with trichloracetic acid, phosphorus determinations (free and total phosphorus) are made in duplicate on both urinary samples, a modified Briggs method being used. The "normal" urinary excretion of phosphorus in milligrams per hour is calculated from the values in the first specimen (8:30 to 10:30 A.M.), and it is assumed that this rate would be maintained approximately in the next hour. By subtracting the hourly "normal" value from the amount of organic plus inorganic phosphorus in the hour following the injection, the "excess phosphorus excretion" is obtained. Comparisons have been made, in 44 cases of renal disease, between this test and the urea concentration and phenolsulphonephthalein tests. The information provided by the glycerophosphate test has been everywhere at least as valuable as that derived from the other two tests, and in certain instances, where there has been a divergence between them, the glycerophosphate test has agreed most closely with the clinical observations.

OVA: Demonstration of, in Feces, Garin, Doubrow, and Mounier. Lyon Med. 141: 341, 1928.

A modification of Telemann's method for the concentration of helminth eggs is proposed. The new method is rapid and has a very high coefficient. It does not cause shriveling of the eggs. The procedure is as follows: To 30 c.c. of hydrochloric acid sulphuric ether is added in small quantities in a graduated flask of 120 c.c. which is constantly shaken under a stream of cold water. A limpid homogeneous mixture forms at last. The addition of ether is continued, the flask being kept continually shaken under the stream of cold water until the flask is filled. The liquid now separates into two layers, a lower clear layer and an upper layer which also clarifies rapidly. It is necessary to shake the reagent when used. A portion of feces is emulsified in from 5 to 20 times its volume of water. This is allowed to sediment for a minute to allow the larger particles to sink. About 10 c.c. are then poured into a centrifuge tube. Three or 4 c.c. of the reagent is added and the tube is vigorously shaken. When the contents are thoroughly mixed about 30 turns in the centrifuge separate the fluid into three distinct layers. The bulk of the contents of the tube is thrown out and the last 2 or 3 drops which remain behind are examined under a coverslip. The procedure, including the examination of the preparation, occupies five minutes only. It is exceptional to find vegetable débris but muscle fiber fragments when badly digested may occur in quantity in the preparation.

GRAM STAIN: A Quick Method for Staining Gram Positive Bacteria In Tissues, Lillie, R. D. Arch. Path. 5: 828, 1928.

Paraffin sections of about 5 microns thickness or frozen sections fixed to the slide by the celloidin method are brought down to water in the usual way.

1. The sections are stained for thirty seconds with ammonium oxalate crystal violet. (Crystal violet 2 gm., 95 per cent alcohol 20 c.c.; 1 per cent ammonium oxalate in distilled water 80 c.c. The preparation is filtered after it has dissolved. It keeps well.) For tubercle

and lepra bacilli, staining for a longer period or heating is often necessary. Heating for ninety seconds on a hot plate at from 50 to 52° C. has been been found satisfactory.

2. The sections are washed in tap water.

3. Lugol's solution I:KI: H_2O = 1:2:100 is applied for thirty seconds.

4. The sections are washed in tap water.

5. Acetone (from dropping bottle) is applied until no more color is removed (about ten to fifteen seconds).

6. The sections are washed in water (they should not be allowed to dry after the acetone is applied.

7. They are counterstained with 0.5 per cent safranine in water for thirty seconds.

8. They are washed in water.

9. They are dehydrated and differentiated with acetone. A certain amount of red comes out in this process, leaving the cell nuclei deep red, and the cytoplasm pink.

10. They are cleared with xylol (in moist weather it may be necessary to blot off the xylol once with filter paper as the acetone is quite hygroscopic).

Gram positive bacteria are blue-black; gram negative organisms red; cell nuclei deep red; cytoplasm of lymphocytes and plasma cells moderately deep pink; cytoplasm of other cells, pale pink; fibrin, pale pink; collagen, usually pink, sometimes light violet in dense bundles.

SPINAL FLUID: Effect of Storage (Icebox) On Cell Count In Pathologic Cerebrospinal Fluid, Novick, N. Arch. Neurol. & Psychiatr. 21: 658, 1929.

In a study of the effect of storage on the cell count in fifteen spinal fluids kept at ice box temperature (about 8° C.), a general tendency for the cells to diminish gradually from day to day was found. The loss is apparently due to the lytic effect of the spinal fluid itself, as was evidenced by microscopic study.

Twenty-four hours' storage at ice box temperature does not invalidate the clinical significance of the cell count.

TUBERCLE BACILLUS: Culture of, Lichtenstein, C. Centralbl. Bakteriol. 108: 239, 1928.

Mix 1 or 2 c.c. of sputum or other material with 10 c.c. of 10 per cent HCl, shake for ten minutes and then centrifuge for ten minutes. Inoculate the sediment on the medium of Petragnani prepared as follows. Mix 150 c.c. of milk, 6 gm. of potato meal and a piece of potato the size of an egg, chopped finely. Place in a boiling water-bath and shake for ten minutes, then let stand in the bath for one hour. Cool to 50° and add 4 whole eggs, 1 egg yolk, 12 c.c. of glycerol; and 10 c.c. of 2 per cent malachite green. Shake vigorously and filter through gauze. Tube and sterilize at 80° for twenty minutes on three consecutive days.

TUMORS: Examination of Fluids for Tumor Cells, Zemansky, A. P. Am. J. M. Sc. 175: 489, 1928.

The technic is a very simple one. The fluid in a large Erlenmeyer flask is allowed to settle by gravity, by standing overnight. The clear upper layer is poured off and as large a quantity of the cloudy sediment as can be conveniently handled is centrifuged in a wide tube (capacity 50 c.c.), tapering to a point. This is centrifuged at moderate speed for at least twenty minutes. The supernatant fluid is poured off without disturbing the sediment, and the tube is filled with 10 per cent formalin. This is allowed to stand for twenty-four hours, when the formalin is poured off. By this time, the sediment is sufficiently hardened to be removed from the tube without falling to pieces. It is gently separated from the tube with a thin blade, lifted out with forceps, and cut from above downward, in order to obtain all layers of the sediment.

This is important, as tumor cells may be missed by cutting only the top or bottom layers. The two halves are then hardened in graded alcohols, run through chloroform paraffin, embedded in paraffin, cut, and stained by hematoxylin and eosin or hematoxylin alone.

ANTHRAX: New Method for Obtaining High Titer Diagnostic Precipitin Sera, Rosenberg, B. and Romanow, D. Centralbl. Bakteriol. 110: 102, 1929.

Wash off a ten to twelve hour agar growth at 33 to 34° in 10 c.c. of normal saline. Add 100 c.c. of distilled water, 20 c.c. of saturated sodium chloride solution, and 6 to 7 drops of acetic acid 50 per cent. Heat in the Arnold twenty to thirty minutes. Filter, wash coagulum in alcohol and dry. Grind in a mortar with 2 c.c. of normal saline, let stand about ten minutes, and inject intravenously.

SPIROCHETES: Method for Culture of Sp. icterohaemorrhagiae, Fukushima, B. Sc. Rept. Gov. Inst. Infect. Dis., Tokyo: 6: 545, 1927.

Citric acid	0.1
Calcium chlorate	0.1
Sodium hydrate	5.5
Tap water	100.0 c.c.

This mixture is sterilized for one hour at 100° C.; 0.3 gm. saccharose is then dissolved in a little distilled water, heated for ten minutes to 100° C. and added to the above solution. Eighty c.c. of the resulting medium are then mixed with 20 c.c. of inactivated horse serum, the resulting P_H being 7.4 to 7.6; the mixture is then run into test tubes, 10 c.c. in each.

Citric acid	0.1
Calcium chloride	0.1
Sodium hydrate	5.5
Glucose	0.2
Tap water	100.0 c.c.

After sterilization for one hour at 100° C., 80 c.c. of this solution are mixed with 20 c.c. of inactivated horse serum and divided into test tubes as in the case of the first medium.

The tubes containing the media are heated for one hour at 56° C., and then one drop of infected guinea pig blood is added to each tube; they are then incubated at 37° C. for forty-eight hours. For making subcultures one drop of normal guinea pig blood is added to each tube, and then 0.1 c.c. of the culture. For making plate cultures the first medium is used, but 75 c.c. of the mixture is mixed with 25 c.c. of horse serum. After heating to 56° C. for one hour one drop of normal guinea pig blood is added to each tube, and these left for twenty-four hours for the fibrin to precipitate. The clear supernatant fluid is then poured on agar plates (without bouillon or peptone) in the proportion of about 1 to 5. The culture of spirochetes, preferably after two or three passages, is then sown on the surface of the plate, which is then grown for four days at 37° C. and afterward at 30° C.

TUBERCLE BACILLUS: A New Stain For, Ransom, C. G. J. Tenn. Med. Assn. 21: 10, 1929.

100 c.c. of distilled water.
10 c.c. of N/10 NaOH or 1 c.c. of N/1 NaOH.
4 per cent or 4 gm. of safranine.
Dissolve well.
The stain is applied and steamed for two minutes.

AMEBAE: Stained Preparations in the Study of Amebic Infections, Riley, W. A. Minn. Med. 12: 65, 1929.

The following technic is advocated:

Schaudinn's fluid:

Saturated aqueous solution of corrosive sublimate	60 c.c.
95 per cent ethyl alcohol	30 c.c.
Glacial acetic acid	3 c.c.

It should be heated until steam is given off (60° to 70° C.) immediately before using. Handling slides with forceps or allowing metal to come in contact with the solution in any way must be avoided or precipitates will ruin the preparations.

Technic:

1. By use of an applicator stick, or a match, smear the fresh fecal material over two-thirds of a thoroughly clean slide. The smear should be thicker than for ordinary bacteriologic work and must not be allowed to dry at any stage until finally mounted in balsam.
2. Fix in the warm Schaudinn's fluid fifteen minutes.
3. Rinse in 50 per cent alcohol three to five minutes.
4. Transfer to 70 per cent alcohol to which has been added enough tincture of iodine to give a bright straw color, ten minutes. If the fluid is bleached renew it.
5. Harden in 95 per cent alcohol, one hour or more.
6. 70 per cent alcohol, five minutes.
7. 50 per cent alcohol, five minutes.
8. Rinse in distilled water.
9. Mordant in a 4 per cent solution of clear violet crystals of iron alum (ammonio-ferric sulphate) in distilled water, six hours.
10. Rinse in water.
11. Stain in 0.5 per cent ripened solution of hematoxylin in distilled water, six hours to overnight.
12. Rinse in water.
13. Differentiate in 2 per cent iron alum solution, controlling by rinsing in water and examining under the microscope from time to time. The background should be grayish and it is desirable to differentiate the slides to a slightly different degree, since it is not feasible to search long for cysts as controls.
14. Wash in running water, twenty minutes.
15. Dehydrate in 50, 70, 95 per cent and absolute alcohol, five minutes each.
16. Clear in zylol, five minutes.
17. Mount in Canada balsam.

The periods suggested for mordanting and staining are not fixed, but usually work out conveniently in practice and give excellent results. The whole process may be greatly reduced, even to the extent of the mordanting for a half hour and staining for an hour, but this short method is more difficult to control.

The properly prepared slide is not only valuable for immediate diagnostic purposes, but affords opportunity for careful, unhurried study. Moreover, it constitutes an important part of the permanent record of the case.

BLOOD FAT: Demonstration of, in Osteomyelitis, Nakata, M. Deutsche. Ztschr. f. Chir. 213: 132, 1928.

Place 0.5 to 1 c.c. of blood from a vein in an alcoholic solution of Sudan III and examine microscopically after five minutes.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Technique of Contraception**

A FULL presentation of the subject by the director of the Clinical Research Department of the American Birth Control League.

The first section is devoted to quotations representing the expression of opinion of prominent persons such as Havelock Ellis, William A. Pusey, Lord Dawson of Penn, Sir Arbuthnot Lane, Prince A. Morrow, Abraham Jacobi, Jos. B. DeLee, Adolphus Knopf, Charles E. De M. Sajou, Clarence C. Little, Raymond Pearl, and others including members of the clergy.

There follows a chapter on the medical indications for contraception, the physiology of impregnation and then a section devoted to a critical clinical study of all the contraceptives that have been recommended. The writer next describes the results of comparative studies made under the direction of the Birth Control League and presents his conclusions concerning the most efficient contraceptive.

The subject is treated judiciously but thoroughly.

The second portion of the book deals with the accomplishment of the social agencies that are interested in eugenics, birth control, and similar aspects of the general subject. The last chapter presents rather briefly the present legal status of contraception in the different states.

The book is sold only to physicians and may be recommended as being authoritative and informative.

Textbook of Clinical Neurology†

ALL good books on the same medical subjects cover essentially the same field. Their differences lie in their manner of presentation and interpretation and, depending upon these factors, one or another of them is especially appropriate for this or that person whose interest in the subject is from this or that angle or viewpoint.

Neustaedter has written his work primarily for the medical man who needs a reference volume when he runs across neurologic problems. The logical basis for the author's presentation and one in which it differs from most other volumes on neurology is then, first, his classification in a general way under broad objective findings such as the spastic paralyses, the flaccid paralyses, the ataxias, tremors and spasms, the trophic disorders, the vasomotor disorders and functional neuroses; and second, his presentation under each individual subject of symptomatology prior to other discussion. Thus, the practitioner finding

*Technique of Contraception. The Principles and Practice of Anti-Conceptional Methods. By James F. Cooper, M.D., Medical Director of the Clinical Research Dept. of the American Birth Control League, Formerly Professor of Clinical Surgery, Foochow Union Medical College, Foochow, China; and Clinical Instructor in Obstetrics, Boston University Medical School. Cloth. 271 pages. Day-Nichols, Publishers, New York, N. Y.

†Textbook of Clinical Neurology for Students and Practitioners. By M. Neustaedter, M.D., Ph.D. Introduction by Edward D. Fisher, M.D. 228 illustrations, some in colors. 602 pages. F. A. Davis Co., Philadelphia, 1929.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

a general type of neurologic lesion may first go through that subject to see whether the symptomatology and gross findings correspond to those of his case, and when he finds the appropriate one or more examples he may then study these in greater detail.

The first ninety pages describe in all necessary detail methods of neurologic examination. The work is unusually well illustrated. The volume should find a welcome place on the reference shelves of internists, and clinicians.

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*Stedman's Medical Dictionary**

A STANDARD medical dictionary brought strictly up-to-date. We do not envy the lexicographer who must keep abreast of all of the new terms offered for adoption in the medical sciences. His reading must be wide, his understanding deep and his discrimination good.

This volume may be recommended as a standard reference dictionary.

Practical Clinical Laboratory Diagnosis†

THIS, the third edition of a well-known and deservedly popular book, has been so extensively rewritten and revised as to be largely a new text.

As is well known it is not intended primarily for the laboratory worker, but for the clinician who does his own laboratory work. As a guide to what should be done and how to do it, it well fulfills its purpose.

The reader is not confused with a multiplicity of methods but directed in the use of those considered most suitable in the light of the author's experience.

The table of leucocytic reactions in disease, that concerned with bacteria, should prove useful to clinicians.

The book is profusely illustrated with microphotographs, drawings, and colored plates of extremely practical type.

One criticism may be made, in this reviewer's estimation: The book is obviously intended for the use of clinicians unskilled in laboratory procedures. To entrust to unskilled hands the serologic study and diagnosis of syphilis by means of a single test, a precipitation reaction, no matter how simple or how excellent in the hands of trained serologic workers, seems ill-advised, at least, until clinicians are more generally and thoroughly familiar with the pitfalls, complexities, and the inherent vagaries of the serology of syphilis than they are at present.

Handbook of Bacteriology‡

THIS is the second edition of a well written text intended especially for the use of students and practitioners of medicine.

It has been extensively revised and rewritten in order to conform to the information concerning this subject which has accumulated in the four years since it was first published.

It reflects very ably the modern outlook upon bacteriology and is in every way suited for the purpose for which it is intended.

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*A Practical Medical Dictionary. By Thomas Lathrop Stedman, A.M., M.D., Editor of *Twentieth Century Practice of Medicine* and of the *Reference Handbook of the Medical Sciences*. Formerly Editor of the *Medical Record*. Tenth, Revised Edition. Illustrated. 1194 pages. Flexible Fabricoid. William Wood & Company, New York.

†Practical Clinical Laboratory Diagnosis. By Charles C. Bass, Dean, Medical Department, Tulane University and Foster M. Johns, Assistant Professor of Medicine, Tulane University. Cloth, 187 pages, 139 illustrations and 6 color plates. Williams and Wilkins Co., Baltimore, Md.

‡Handbook of Bacteriology. By Joseph W. Bigger, Professor of Bacteriology and Preventive Medicine, University of Dublin. Ed. 2. Cloth, 82 illustrations, 452 pages. William Wood & Co., New York.

*Report of the International Conference on Cancer**

THIS volume, representing as it does the opinions of an exceedingly catholic group of international experts, may be said to represent a cross-section of the facts concerning cancer now accepted and also concerning those still under investigation.

It well repays a careful reading by the clinician as well as by the special worker.

The section on the etiology of cancer, while summarizing such facts as are known and adding others, as yet cannot present a solution to the problem.

The section on the relative values of surgery and radiation in the treatment of cancer is of great interest. The views of the enthusiast, the pessimist, and the conservative are all represented with those of the conservative carrying the day.

Of equal interest is the section on treatment by chemotherapy with special reference to the use of lead.

The cumulative experience thus gathered leads to the inevitable conclusion that the lead treatment sponsored by Blair Bell is without specific effect on malignant neoplasms.

In the section on pathology there is a wealth of information. This is true also of the section on diagnosis in which, among other things, various proposed serologic methods are discussed.

A section on radiology, one on the geographic and racial prevalence of cancer, and one on public action with regard to cancer conclude a volume of great interest to all who are interested, and who is not? in this most important subject.

Experimental Bacteriology and Infectious Diseases With Especial Reference to Immunity†

THIS is a new edition of the well known, standard German work which has already been translated into twelve other languages. It is a treat to turn the pages and look at the marvelous illustrations, 118 colored plates and 200 black and white text figures, many of the illustrations of an excellence found only in German works.

Extensive changes and additions have been made in this edition to include the advances made during the seven years since the previous edition; some of the topics most extensively revised and enlarged being the bacteriophage, tularemia, streptococci in scarlet fever, and the question of herpes and postvaccinal encephalitis. However, the size of the two volumes has not been increased as the authors have been able to throw out an almost equal volume of material.

Americans have always wondered how the Germans find time to read such elaborate works and the authors in the preface bewail the fact that members of the younger generation have not been as anxious to thoroughly ground themselves in their subject by industriously studying the great works as was previously the case, and state that the old spirit must return if the reputation of their professional men is not to suffer.

They also state that the difficulty experienced by authors since the war in being unable to obtain foreign periodicals, with the result that their presentations have not been well rounded has now been largely overcome.

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*Report of The International Conference on Cancer. London, 1928. Cloth, 588 pages. Wm. Wood & Co., New York.

†Experimental Bacteriology and Infectious Diseases With Especial Reference to Immunity. By Dr. W. Koole and Dr. H. Hetsch. Urban and Schwarzenberg, Berlin and Vienna, 1929.

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EDITORIALS

Geographic Variations in the Size of the Red Blood Cell

FIVE million red blood cells are usually taken as the average count of the normal adult male. The count is higher at high altitudes and among certain peoples such as the Scandinavians. Relatively few careful studies have been made to determine variations in the number of erythrocytes in different parts of the United States. Emerson[1] found the mean count of 176 male medical students between the ages of twenty and twenty-five in Baltimore to be exactly 5.0 millions per cmm. The average of 40 normal men from eighteen to fifty years of age in Kansas City was 4.97 millions.[2] Recently comprehensive studies from different parts of this country show widely varying results. In Portland (Oregon), Osgood[3] examined the blood of 137 normal young men between the ages of nineteen and thirty and found the average red cell count 5.39 millions. Wintrobe and Miller[4] report from New Orleans an average count of 5.85 millions in 100 medical students between the ages of nineteen and thirty.

The studies from Portland, New Orleans, and Kansas City also record the relative volume of packed cells after centrifugation. If corrections are made for differences in technic, the average cell volume is everywhere the same, within the limits of error of determination. The comparative data for normal men between the ages of nineteen and thirty has been summarized by Wintrobe and Miller.[4] All observers find approximately 46.5 c.c. of packed cells per 100 c.c. of blood. Likewise the amount of hemoglobin per 100 c.c. as determined by Van Slyke's method is practically the same. Thus in Portland the figure is 15.76 gm. per 100 c.c.; in New Orleans, 15.87 gm.; and in Kansas City, 15.83 gm. It is apparent the only geographic difference in the erythrocytes is the number, since the total volume of cells and the total hemoglobin is everywhere the same.

This variation in number of red cells is most important since it affects the color and volume index and the average individual cell volume and hemoglobin content. For the indices one must take as normal the hemoglobin in grams and the cell volume in c.c. per 100 c.c. of blood per 5 million cells ("hemoglobin coefficient" and "volume coefficient" of Wintrobe and Miller). It is apparent these coefficients vary directly with the cell count since the hemoglobin content and the total cell volume are constant. In New Orleans the hemoglobin coefficient is 13.65 gm. and the volume coefficient is 39.7 c.c.; in Portland, 14.66 gm. and 42.2 c.c., and in Kansas City, 15.57 gm. and 45.8 c.c. This variation necessitates the determination in every place of the average red cell count and hemoglobin and volume coefficient for accurate calculation of the color and volume indices. Tables, such as those given by Osgood,[5] can be used only where the red cell count is the same as that found in Portland.

The most desirable measure of volume of the red cell is cubic microns. This of course also varies with the cell count when the total volume of cells remains constant. In Portland the average individual cell volume is 84.4 cubic microns, in New Orleans, 79.4 and in Kansas City, 91.6. Similarly the actual hemoglobin content of the average red cell in Portland is 29.2 by 10^{-12} gm., in New Orleans, 27.6 by 10^{-12} gm., and in Kansas City, 31.1 by 10^{-12} gm. So far no studies of red cell diameters have been reported to compare with other cell measurement. In Kansas City[6] the average red cell diameter in normal adults is the same as that found in Boston and generally accepted as the average cell diameter (7.7 microns).

Emmons[7] has recently reported a study of the interrelation of number, volume, diameter, and area of erythrocytes in man, the dog, the rabbit, the cat and the goat. The red cell count of the goat is 15 millions and of the cat, 9.0 millions per cmm., yet the volume percentage of red cells is little different from that of man. The individual cell volume of the goat is 24.6 cubic microns. The cell thickness, however, is much the same as that of man with almost four times the cell volume. The variation in volume is due almost entirely to difference in diameters. Likewise the total cell surface area in square centimeters per cmm. of blood is almost exactly the same in all animals studied. Emmons makes the interesting suggestion that the blood count of any mammal can be determined from data concerning the cell diameter alone.

The observations of Emmons in animals are of great interest in connec-

tion with the demonstration in man of constant cell volume and hemoglobin, even with widely different numbers of cells. They bear out the observations discussed that with an increase in number of cells there is a corresponding decrease in the volume of the average red corpuscle. If normal counts well below 5 millions are found anywhere, it is probable there will be a compensatory increase in the volume of the cell such as is found typically in pernicious anemia. It is hoped that further comprehensive studies will be made elsewhere on the physical constants of the red cell which are receiving increasing emphasis in clinical medicine. Even now the determination of whether a patient is getting sufficient liver in the diet is based on the size of the average red blood cell rather than total red cell count or hemoglobin content.

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—*R. L. H.*

Newer Studies On Pernicious Anemia

THE announcement by Minot and Murphy[1] two years ago that the addition of large amounts of liver to the diet was a specific treatment for pernicious anemia revealed a discovery of extraordinary interest and value.

Since their original publication a voluminous literature has accumulated, all corroborating the results first reported, and leading to the assumption that, if the exact cause of pernicious anemia is as yet debatable, at least a specific means of treatment is at last available, and that there is in the liver an anti-pernicious anemia body which is therapeutically utilizable.

As a result of the studies made of this new and by far the most effective method of treatment yet devised, new interest has awakened in the etiology of pernicious anemia and new theories have been advanced which it is the purpose of this communication to summarize and survey.

The introduction of liver as a dietetic treatment for anemia arose from the experimental observation of Whipple[2] concerning the importance of this organ in the regeneration of blood in experimental chronic anemia, as a result of which he suggested its possible value in the treatment of secondary anemia; adding that "even in the complex anemias (human pernicious anemia, anemia with nephritis, and cancer cachexia) food factors deserve serious consideration in the clinical management of the blood condition."

Modern conceptions have long regarded the essential factor in the production of pernicious anemia as a lack of erythrocyte elements capable of undergoing maturation rather than merely an increased blood destruction.

In view of the newer facts now available it seems quite probable that, as stated by Means and Richardson,[3] "pernicious anemia is in some sense or other a deficiency disease rather than an infectious, toxic or neoplastic one; but it

seems doubtful that pernicious anemia bears more than a slight resemblance to any of the other deficiencies with which we are familiar."

That the absence of some specific substance is an essential factor in the production of the disease is no longer to be doubted but nothing is known as yet of its nature.

Synthesis of liver or liver extract so far has revealed nothing and the work of Castle and Locke,[4] indeed, suggests that in the patient with pernicious anemia the lack of specific substance may be due, in part, at least, to a lack of digestive ability which, perhaps, is necessary to supply elements needed in its synthesis.

Despite the remarkable and even dramatic changes produced in the clinical picture of pernicious anemia following the use of liver diet or liver extract, it is as yet too early to say beyond question that the disease is cured. We do not yet know what may happen when the treatment is discontinued. Certainly it appears, however, that with the continuance of treatment the patient remains clinically and hematologically normal indefinitely. There are still, however, problems requiring study.

Koessler and Maurer,[5] for example, are convinced of the necessity for a high fat content in the diet, opposing the contrary recommendation of Minot and Murphy.

This contention is based upon their studies of the relation of vitamine A deficiency to the production of anemia[6] from which they conclude that:

"1. The fat soluble vitamine A is essential to normal blood regeneration.

"2. The addition of vitamine A to the diet of animals long depleted in their vitamine A reserve brings about rapid formation of new blood cells.

"3. A definite relationship exists between a state of chronic vitamine deficiency and certain anemias in man."

The assumptions made by this theory may be thus stated:

"1. The changes in the bone marrow, blood and intestinal tract are probably due to a chronic deficiency or imbalance in fat soluble vitamines.

"2. The lesions and symptoms of the nervous system so similar to those found in pellagra may be related to a chronic deficiency in vitamine B.

"3. The tendency to hemorrhages, retinal and otherwise, may be due to a long standing deficiency in vitamine C."

They believe, therefore, that the diet should be rich in all vitamines, including liver and kidney as containing the largest amounts of vitamines A and B and also some C, and have proved to their entire satisfaction the efficiency of such a diet in the treatment of pernicious anemia in forty-two cases.

A question of some interest is the relation of liver feeding to the treatment of secondary anemias to which it has naturally been applied.

The original work of Minot and Murphy showed that improvement in secondary anemias treated with liver diet, if it occurred at all, was very slow and very much less marked than that seen in pernicious anemia.

Brill[7] reports a comparative study of ten cases of pernicious anemia and four of severe secondary anemia treated with the Minot-Murphy diet over a period of one year. Two of the latter series were cases of more or less con-

tinuous bleeding from benign lesions of the uterus and rectum, one a case of gastric carcinoma, and in one the anemia was apparently due to syphilis.

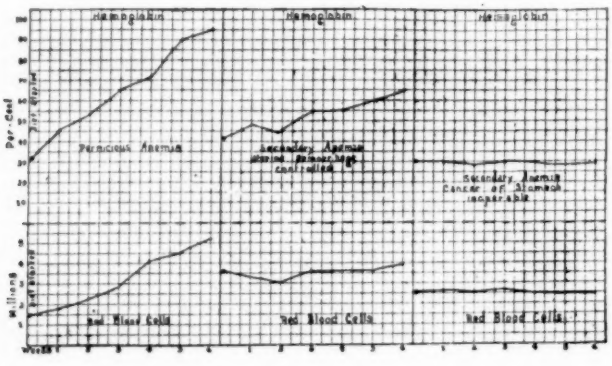
In these groups there were striking differences in the response to treatment. In the pernicious anemias the rise in hemoglobin and red cells was uniform, rapid, and steady. In the secondary anemias the rise was but slight in the cases in which operation had removed the cause before the treatment was begun, and in the case of gastric carcinoma no change at all was seen.

His findings are best seen in the table and chart below.

BLOOD CHANGES IN TEN CASES OF PERNICIOUS ANEMIA AND FOUR CASES OF SECONDARY ANEMIA TREATED BY THE MINOT-MURPHY DIET

	CASE	HEMOGLOBIN PERCENTAGE ADMISSION	HEMOGLOBIN PERCENTAGE DISCHARGE	RED BLOOD CELLS ADMISSION	RED BLOOD CELLS DISCHARGE
Pernicious Anemia	1	26	93	1,280,000	5,000,000
	2	32	95	1,380,000	5,200,000
	3	38	100	1,740,000	5,330,000
	4	30	100	1,360,000	5,120,000
	5	34	100	1,250,000	5,290,000
	6	45	80	1,960,000	3,700,000
	7	81	111	3,710,000	5,010,000
	8	36	85	3,340,000	4,540,000
	9	40	98	1,330,000	4,390,000
	10*	24	65	1,000,000	3,270,000
Secondary Anemia	1	43	62	3,270,000	3,990,000
	2	48	65	3,000,000	3,750,000
	3	20	33	2,080,000	2,660,000
	4	28	28	2,580,000	2,460,000

*This patient remained in the hospital only thirteen days.



In view of these results Brill suggests that it is not at all unreasonable to assume an etiologic relationship between the disease and the diet from which it would follow that it would be comparatively useless in the treatment of anemias of secondary origin.

To this assumption added weight is brought by Faher[5] of the usefulness of liver treatment as a means of identifying the nature of anemias in infancy and childhood.

In three cases in infants, the blood picture in all resembling that of primary anemia, striking and rapid improvement followed the administration of liver extract in two, the third showing no response of any sort but responding promptly to transfusion.

The essential difference, as already stated, seems to be that in secondary anemia there is such fatigue of the hematopoietic system that blood regeneration is imperfect and delayed though all the requisites are present, while in pernicious anemia there is a lack of erythrocyte elements capable of undergoing maturation.

At all events, the common assumption of the past half century that pernicious anemia is a process primarily hemolytic in character must be revised, and we now regard the disease as dependent, not upon blood destruction, but upon inadequate blood formation.

The mechanism involved in the response to liver therapy in pernicious anemia has been carefully studied and reported upon by Minot, Murphy and Stetson[7] as follows:

Both the percentage and absolute numbers of retioculocytes in the peripheral blood at the peak of their rise are related to the level of the red blood corpuscles at the time treatment is begun. These relations may be employed to help determine the potency of material fed a patient.

Up to the peak of the reticulocyte rise the increase in the concentration of total red blood corpuscles may be ascribed almost entirely to the production of reticulocytes when the red blood cells are less than 2.8 million per c.mm.

In cases with over 3 million red blood cells per c.mm., or in cases which have received daily maximal amounts of potent extract for over twelve days, the increase in the total concentration of red blood cells is dependent chiefly on the liberation from the bone marrow of mature corpuscles.

In pernicious anemia with less than 3 million red blood cells per c.mm., the absence of a reticulocyte response renders it exceedingly probable that an impotent extract has been employed. On the other hand if the extract given is known to be potent, and no distinct reticulocyte increase follows, it is very improbable that the patient has pernicious anemia, provided his red blood cells are below 3 million per c.mm.

The number of reticulocytes that appear in the blood at the peak of their rise is roughly proportional to the amount of active principle ingested daily, provided submaximal amounts are fed to patients with less than 2.5 million red blood cells per c.mm. When large amounts are given the reticulocytes are apt to appear sooner and vary more in concentration in different cases with the same erythrocyte level.

In some cases there are probably many primitive cells to be transformed within the marrow and in others relatively few, so that in the latter more reticulocytes would not enter the blood stream even if more active principle were given. Standardized extract from 500 or 600 gm. of liver apparently is sufficient to produce a maximum response of young red blood cells in essentially all cases.

There is a direct relationship between the rate at which the red blood corpuscles increase and the amount of potent material fed, up to a maximum. On the average when liver extract derived from 500 to 600 gm. of liver has been fed daily to patients with less than 2 million red blood cells per c.mm. of blood, it has increased the concentration of these cells about 2.5 million per c.mm. in thirty days. After two months of adequate therapy with liver

extract red blood cell counts in the vicinity of 5 million per c.mm. are to be expected.

The active principle effective in pernicious anemia appears particularly to stimulate the formation of red blood cells. The return to normal of red blood corpuscles usually proceeds more rapidly than that of hemoglobin as the result of treatment with liver, with kidney, or with liver extract.

The hemoglobin increases perhaps more slowly in cases treated with liver extract and a diet poor in sources of iron, and in foods that accelerate hemoglobin regeneration, than when potent extracts are given and the diet is well balanced, and rich in such foods.

It is emphasized that the continued ingestion of some source of the active principle effective in pernicious anemia is necessary to prevent relapse in this disease.

As stated by Means and Richardson, the accumulating facts of the newer studies of pernicious anemia warrant these conclusions:

"1. Pernicious anemia is a chronic disease of unknown etiology, the chief manifestations of which can be held in abeyance as long as adequate amounts of a specific substance contained abundantly in liver and kidney are received.

"2. The supply of this substance can be rendered adequate in persons suffering from the disease by feeding large quantities of natural foods which contain it (liver or kidney), or active fractions thereof, or perhaps also through the provision of the means for natural gastric digestion.

"3. Shortage of the specific substance is clearly responsible for the abnormalities in the liver and blood. The cause of the shortage is not completely known. Castle's work suggests that the gastric defect may play an important rôle.

"4. Whether the normal human being requires any extraneous supply of the specific substance or can synthesize what he needs is not yet known. The problem of whether the active substance now found is in the state that the body needs or is merely a necessary building stone remains unsolved."

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—*R. A. K.*

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Side Lights of the Convention

The Eighth Annual Convention of the American Society of Clinical Pathologists was held in Portland just previous to the meeting of the American Medical Association and again registered a very successful meeting.

There was a cordial reunion of all the old war horses of the early days together with many enthusiastic recent members. The informal chats in the lobbies between the scientific sessions or at meals formed a very delightful feature of the proceedings. Every visitor vowed that nothing would prevent him from attending next year's convention. Many of the members were accompanied by their wives. There was also a good sprinkling of lady pathologists who participated in the proceedings.

All the past presidents with two exceptions were among those present. It was an inspiring sight to see them sitting at the speaker's table on the evening of the banquet.

No inconsiderable credit for the success of the convention is due to the wonderful preparations made by the Local Committee, especially Drs. Foskett and Manlove, which insured us smooth functioning of the machinery. Nothing had been left undone. The entertainment of the members was one that will long be remembered by those who participated in the automobile ride on the Columbia Highway and around Mount Hood. The pleasant incidents of the trip will be the topic of conversation by the winter fireside, especially among those who took snapshots of the scenic wonders encountered.

The visiting ladies were entertained by a drive around the city and a bridge luncheon at the home of Mrs. H. H. Foskett, wife of our Chairman of the Committee on Arrangements.

A number of the members en route to or from the convention took advantage of the opportunity to visit the Office of the Society at Denver and were entertained by the local pathologists. Among those who registered were Dr. and Mrs. J. H. Black of Dallas, Texas, Dr. M. D. Bell and Dr. J. E. Robinson also of Dallas, Dr. G. B. Kramer, Youngstown, Ohio and Dr. F. B. Johnson of Charleston, South Carolina.

The scientific program was all that could be desired and constituted a treat for the members and visiting scientists. The symposium on Undulant Fever elicited considerable discussion.

The Ward Burdick Prize for the best work in research was awarded to Dr. Walter M. Simpson of Dayton, Ohio, for his pioneer investigations in Tularemia. The American Medical Association had presented Dr. Simpson with a prize for research along the same lines a year ago. It was the unanimous resolve of the Committee on Research of the American Society of Clinical Pathologists that the first medal be given to Dr. Simpson. The medal was presented to the recipient by the President, Dr. F. W. Hartman, who in a few well-chosen remarks complimented him on his labors. Dr. Simpson was the most surprised man in the audience and though usually fluent and ready in speech was too stunned to reply except to express his thanks. The presentation of this medal will undoubtedly act as a stimulus to our Fellows to emulate the example of Dr. Simpson.

The Round Table Discussion devoted to economic matters evoked the usual lively and spirited discussion permitting the members to let off high pressure emotion and get grouches out of their system in the course of their jeremiads on some deplorable phases of the practice of clinical pathology. This year, however, was marked by a greater optimism and prospects for improvement in conditions.

The business session was marked by fine harmony and unanimity. The following officers were elected:

President-Elect, Dr. Kenneth M. Lynch, Charleston, South Carolina.
Vice-President, Dr. H. H. Foskett, Portland, Oregon.

Members of the Executive Committee:
Dr. F. W. Hartman, Detroit, Michigan
Dr. A. S. Giordano, South Bend, Indiana

Members of the Board of Censors:
Dr. F. H. Lamb, Davenport, Iowa
Dr. Warren T. Vaughan, Richmond, Virginia

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Dr. Alvin G. Foord, Buffalo, New York
Dr. C. Y. White, Philadelphia, Pennsylvania
Dr. E. S. Maxwell, Lexington, Kentucky
Dr. Wm. Thalhimer, Chicago, Illinois

Considerable scrutiny was made of the applications of the new members; each one having been carefully gone over and investigated by the Board of Censors and considered in the executive session. The following clinical pathologists were elected to membership:

Fellows:
Dr. Arthur Amolsch, Detroit, Michigan
Dr. O. A. Brines, Detroit, Michigan
Dr. Henry T. Brooks, New York City, New York
Dr. Frank L. Kelly, Philadelphia, Pennsylvania
Dr. Frank P. Hunter, Lafayette, Indiana
Dr. Theodore Helmbold, Pittsburgh, Pennsylvania
Dr. Walter G. Bain, Springfield, Illinois
Dr. Hugh G. Jeter, Oklahoma City, Oklahoma
Dr. Harry Langdon, Indianapolis, Indiana
Dr. Richard C. Henderson, Perry Point, Maryland

Reinstatement:
Dr. W. W. Hall, Mercy Hospital, Watertown, New York

Associate Membership:
Dr. Willa M. F. Davis, Washington, D. C.

Corresponding Membership:
Secretary of the British Pathologists Association.